

Figure S1. A) Simulated annealing Fo-DFc difference electron density (contoured at 2.5σ ; green) of ChivoA, with the actin surface coloured according to the surface residue charges. B) Crystal-lattice contacts at the ChivoA binding site. C) Crystal contacts between the actin-ChivoA complexes occur between the two adjacent ChivoA molecules, while neighbouring actin molecules in the crystal lattice contact do not form stable interfaces (PISA analysis). D) Schematic diagram of the interactions between actin and ChivoA. Hydrophobic interactions are indicated by the red half-circles. The 2D-plot was prepared with LigPlot. (REF: Laskowski R A, Swindells M B (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.*, 51, 2778-2786. [PubMed id: 21919503])

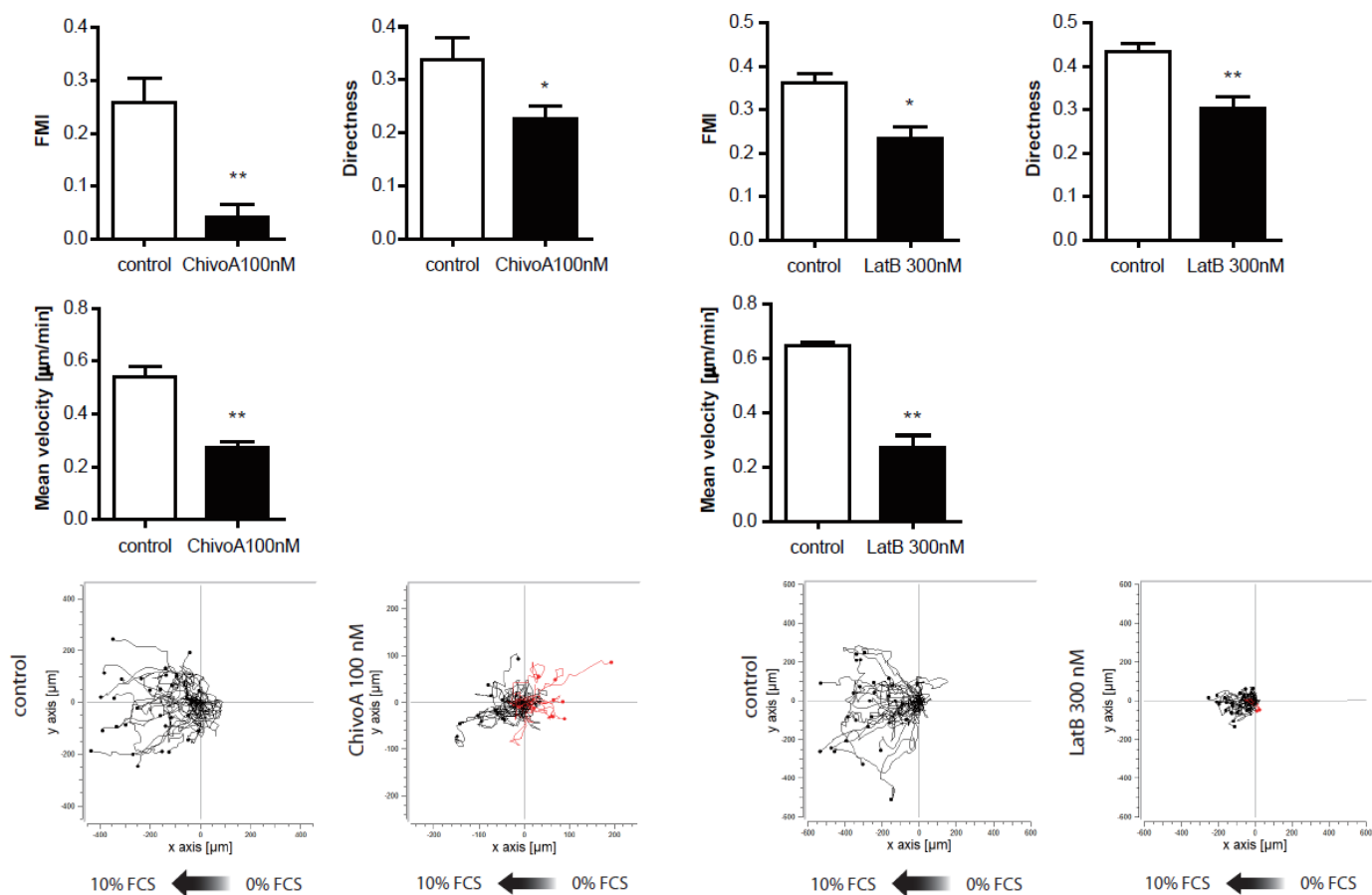


Figure S2: For 2D-chemotaxis assays HUVECs were seeded and treated with indicated concentrations of ChivoA or LatB. Time-stacks of cell migration images were taken every 10 min for 21 h. Cell migration parameters (X-forward migration index, directness and mean velocity) were analyzed with ImageJ and are shown in the upper panels. Data are presented as mean \pm SEM, $n = 3$, $*p < 0.05$ using Kruskal-Wallis test. Lower panels: Tracking of one representative experiment each is shown, red lines: trajectories of cells not following the gradient.

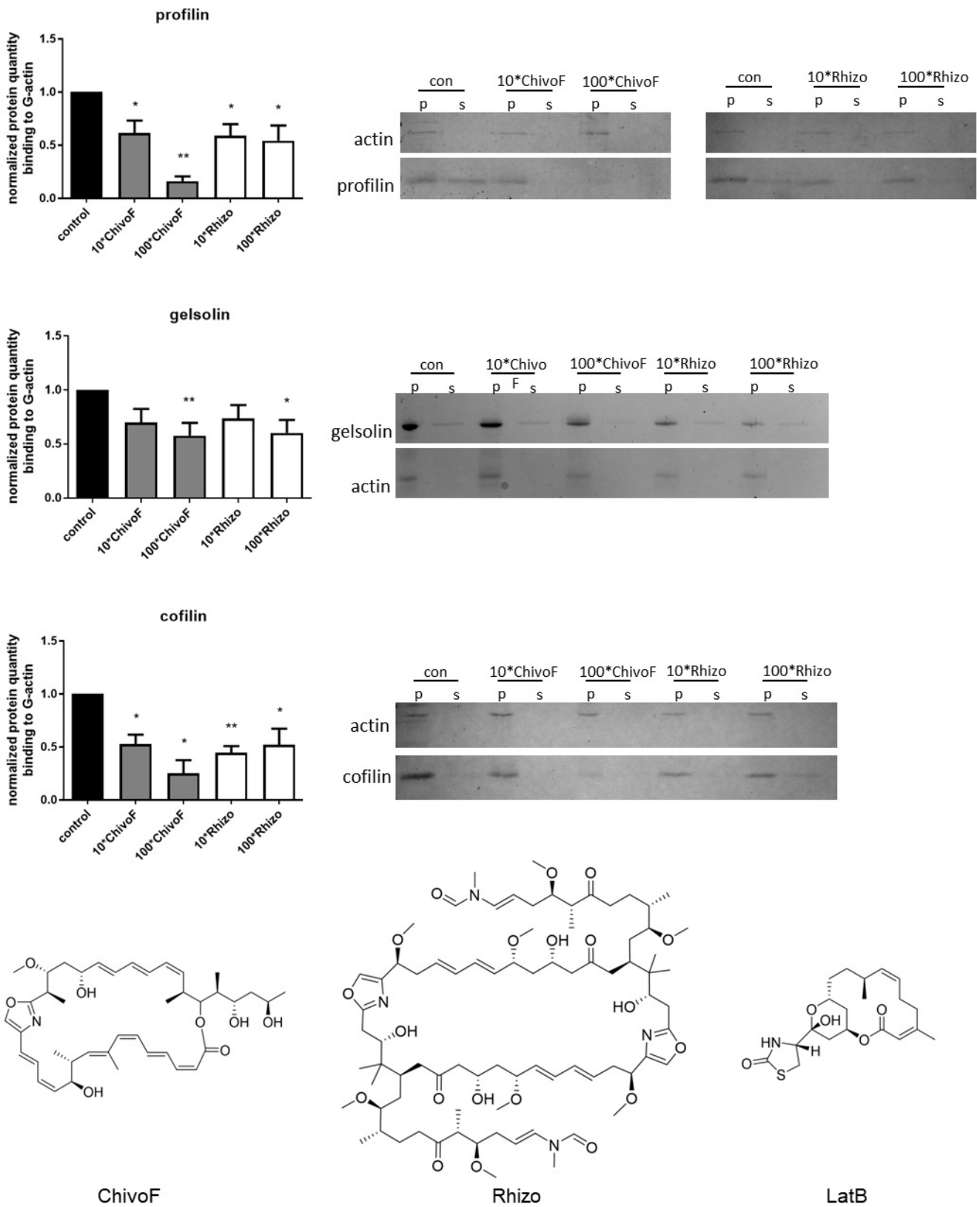


Figure S3: Rhizopodin (Rhizo) and chivosazole F (ChivoF) both compete with binding of profilin (upper panels), gelsolin (middle panels) and cofilin (lower panels) to G-actin. Left panels: Data are presented as mean \pm SEM, $n = 3$, $*p < 0.05$ using Kruskal-Wallis test. Right panels: Images of the respective gels. The gray value of the band of the respective protein in controls was defined as 1.0. The other band were normalized to control accordingly. Bottom part: chemical structures of ChivoF, Rhizo and LatB (from left to right)

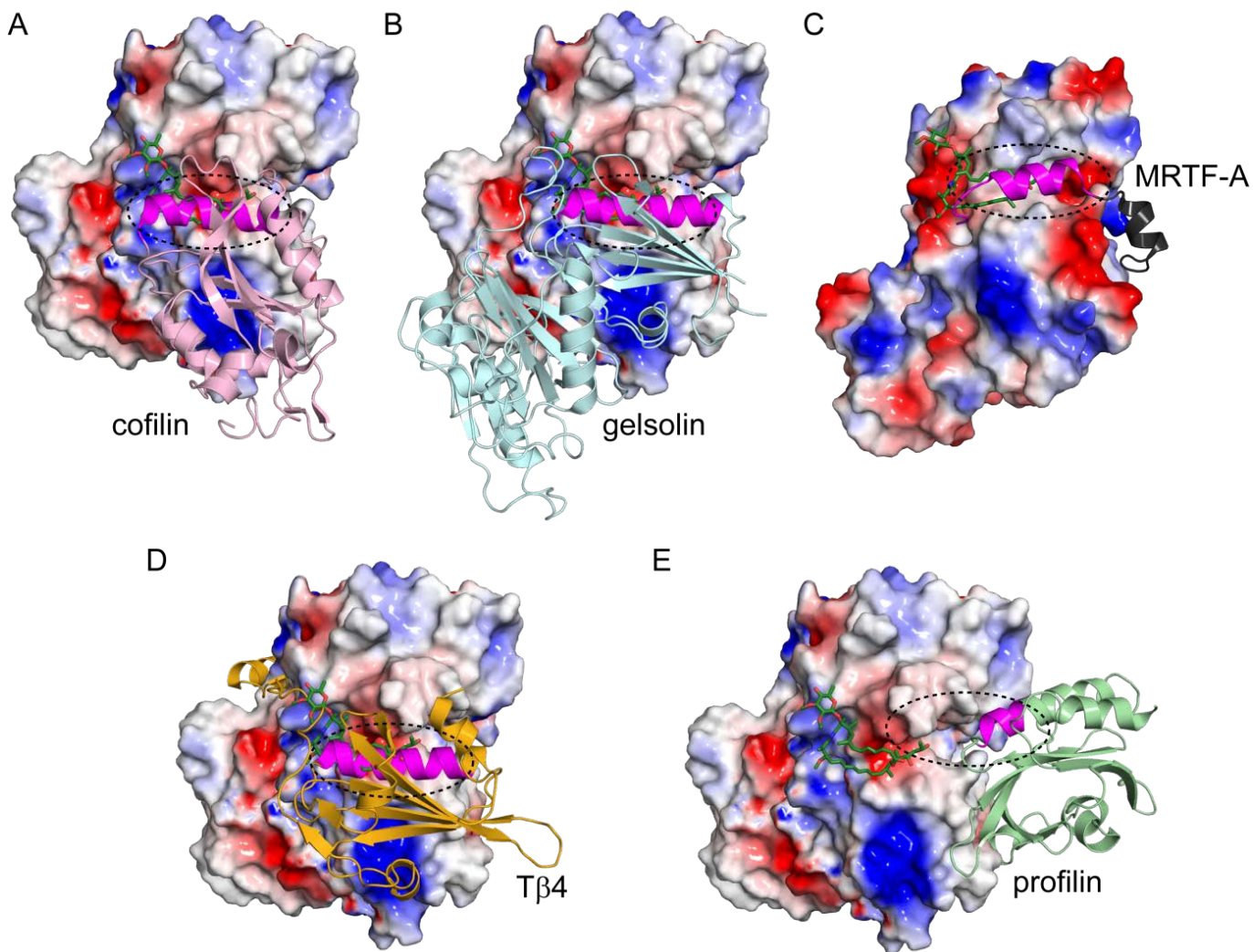


Figure S4: Comparison of ChivoA binding site and the interaction interface of actin binding proteins. A) cofilin (light pink; PDB code 3J0S), B) gelsolin (cyan; PDB code 1H1V), C) MRTF-A (grey, PDB code 2V52), D) thymosin- β 4 (golden; PDB code 1T44) and E) profilin (light green; PDB code 2BTF). The actin is shown as surface representation coloured according to the surface charge. ChivoA is depicted as green stick model. The amphiphilic helices of the actin-binding proteins, which pack into the target binding groove, are highlighted in pink.

Table S1. Data collection and refinement statistics. Statistics for the highest-resolution shell are shown in parentheses.

Actin-ChivosazoleA	
Wavelength	0.873
Resolution range	49.2- 2.4 (2.49-2.4)
Space group	P 2 ₁
Unit cell	72.5 78.6 77.1 β =115.1
Total reflections	108,620 (10,337)
Unique reflections	30,437 (2,925)
Multiplicity	3.6 (3.5)
Completeness (%)	98.8 (95.6)
Mean I/sigma(I)	7.0 (0.98)
Wilson B-factor	45.1
R-merge	0.13 (1.03)
R-meas	0.16 (1.21)
R-pim	0.08 (0.63)
CC1/2	0.99 (0.47)
CC*	0.998 (0.797)
Reflections used in refinement	30,436 (2,924)
Reflections used for R-free	1,522 (146)
R-work	0.215 (0.333)
R-free	0.254 (0.308)
CC(work)	0.954 (0.658)
CC(free)	0.931 (0.506)
Number of non-hydrogen atoms	5,853
macromolecules	5,619
ligands	188
solvent	46
Protein residues	718
RMS(bonds)	0.010
RMS(angles)	1.51
Ramachandran favored (%)	98.0
Ramachandran allowed (%)	1.99
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	1.8
Clashscore	2.9
Average B-factor	60.4
macromolecules	60.8
ligands	51.0
solvent	42.0
Number of TLS groups	2

Table S2: List of genes significantly regulated in HUVECs by treatment with 20 nM ChivoA for 4h. Orange: down regulated genes, green: upregulated genes, NA: non protein coding genes.

ENSEMBL	symbol	log2 fold change	adjusted p value
ENSG00000278806	NA	-4,711252094	0,00139022
ENSG00000234576	NA	-4,564728721	0,00011785
ENSG00000225718	NA	-3,510179757	0,00229876
ENSG00000231034	NA	-3,41544532	0,00626786
ENSG00000229735	NA	-3,127949743	0,01961863
ENSG00000166165	CKB	-2,704005339	9,92E-05
ENSG00000236571	NA	-2,654571366	0,04171752
ENSG00000233829	NA	-2,651032822	0,05329787
ENSG00000268391	NA	-2,381502657	0,03993551
ENSG00000251340	MTCYBP35	-2,175472007	0,04915397
ENSG00000266920	NA	-1,851983819	0,03641245
ENSG00000156466	GDF6	-1,765741656	8,92E-08
ENSG00000255266	NA	-1,676356622	0,05183135
ENSG00000109846	CRYAB	-1,669887718	0,00381191
ENSG00000078401	EDN1	-1,589883974	3,40E-39
ENSG00000165702	GFI1B	-1,569041341	0,03693842
ENSG00000118523	CTGF	-1,485906037	1,37E-38
ENSG00000125378	BMP4	-1,476586972	2,56E-13
ENSG00000107984	DKK1	-1,451643699	8,46E-19
ENSG00000235387	SPAAR	-1,345679574	1,47E-07
ENSG00000144647	POMGNT2	-1,296480359	0,00338969
ENSG00000146674	IGFBP3	-1,271827367	0,04492982
ENSG00000148677	ANKRD1	-1,264103878	7,10E-22
ENSG00000243256	NA	-1,176852375	0,00091206
ENSG00000081692	JMJD4	-1,134625587	0,00650101
ENSG00000176697	BDNF	-1,12664306	0,00611232
ENSG00000163739	CXCL1	-1,106669894	6,90E-08
ENSG00000159399	HK2	-1,100730212	0,01758736
ENSG00000142871	CYR61	-1,084531632	2,88E-23
ENSG00000120217	CD274	-1,042662377	0,05106095
ENSG00000226915	NA	-1,018300709	0,04277393
ENSG00000112029	FBXO5	-0,977233153	0,0032306
ENSG00000235045	NA	-0,954167559	0,01668169
ENSG00000117152	RGS4	-0,932751547	4,15E-08
ENSG00000151093	OXSM	-0,930204096	0,02212105
ENSG00000226860	NA	-0,921720286	0,01930647
ENSG00000104833	TUBB4A	-0,920009066	0,02173595
ENSG00000164815	ORC5	-0,898115897	0,01709753
ENSG00000178882	RFLNA	-0,891361029	0,00022723
ENSG00000157168	NRG1	-0,767036575	0,00232789
ENSG00000132436	FIGNL1	-0,736941866	0,01385136
ENSG00000115875	SRSF7	-0,716940606	1,32E-15
ENSG00000183741	CBX6	-0,713469689	0,00359312
ENSG00000092098	RNF31	-0,708179646	0,04327808

ENSG00000127824	TUBA4A	-0,704994591	0,02647267
ENSG00000179431	FJX1	-0,68995993	0,01579507
ENSG00000182253	SYNM	-0,688613397	0,01951484
ENSG00000146834	MEPCE	-0,686588486	0,02601277
ENSG00000214870	NA	-0,682478229	0,00570223
ENSG00000099860	GADD45B	-0,678618177	0,01501352
ENSG00000144120	TMEM177	-0,67293138	0,00121859
ENSG00000122971	ACADS	-0,665025042	0,01780188
ENSG00000214289	NA	-0,65332803	0,00238371
ENSG00000189369	GSPT2	-0,646458553	0,01410789
ENSG00000155438	NIFK	-0,635431774	4,79E-05
ENSG00000137267	TUBB2A	-0,605154126	3,09E-07
ENSG00000244756	NA	-0,604714512	0,01422129
ENSG00000099260	PALMD	-0,585014555	1,28E-05
ENSG00000119408	NEK6	-0,572140637	0,00839702
ENSG00000188229	TUBB4B	-0,558492083	2,43E-10
ENSG00000228495	NA	-0,555991338	0,00017305
ENSG00000137285	TUBB2B	-0,555309058	1,21E-07
ENSG00000130340	SNX9	0,747090146	5,69E-11
ENSG00000275023	MLLT6	0,747468252	0,01765458
ENSG00000166510	CCDC68	0,748366521	0,00787823
ENSG00000130513	GDF15	0,749992949	3,82E-25
ENSG00000173575	CHD2	0,752044832	2,49E-08
ENSG00000088826	SMOX	0,761299352	0,00209826
ENSG00000257242	LINC01619	0,761719758	1,23E-13
ENSG00000144560	VGLL4	0,769596185	0,00090707
ENSG00000023287	RB1CC1	0,778228714	3,01E-05
ENSG00000215788	TNFRSF25	0,794904425	6,90E-06
ENSG00000188559	RALGAPA2	0,796218801	0,02130162
ENSG00000174718	KIAA1551	0,802010826	0,00870919
ENSG00000157514	TSC22D3	0,806541361	0,02471592
ENSG00000167106	FAM102A	0,811860557	0,01293999
ENSG00000168916	ZNF608	0,82181994	0,03610715
ENSG00000101445	PPP1R16B	0,826356979	0,00650101
ENSG00000159167	STC1	0,833760609	0,0019435
ENSG00000100784	RPS6KA5	0,885357525	0,00575559
ENSG00000245532	NEAT1	0,891768378	1,02E-21
ENSG00000140199	SLC12A6	0,893582467	0,02629094
ENSG00000162852	CNST	0,896329093	0,01267482
ENSG00000152518	ZFP36L2	0,91337122	2,86E-13
ENSG00000119138	KLF9	0,916987276	0,00128791
ENSG00000109787	KLF3	0,951547018	8,52E-05
ENSG00000161940	BCL6B	0,970565409	4,99E-14
ENSG00000127528	KLF2	0,97722175	3,91E-13
ENSG00000073756	PTGS2	1,01561324	2,43E-10
ENSG00000100916	BRMS1L	1,039595297	0,03534367
ENSG00000136630	HLX	1,067377216	2,26E-08

ENSG00000164463	CREBRF	1,149750682	0,00069663
ENSG00000158859	ADAMTS4	1,178642387	4,23E-23
ENSG00000183337	BCOR	1,183057282	0,00381859
ENSG00000138061	CYP1B1	1,271957146	2,58E-06
ENSG00000154734	ADAMTS1	1,277419617	8,97E-20
ENSG00000246451	NA	1,306544402	0,02269402
ENSG00000136826	KLF4	1,407640121	0,00367329
ENSG00000154240	CEP112	1,572562073	0,01110282
ENSG00000266010	GATA6-AS1	2,120515199	0,03267041
ENSG00000134323	MYCN	2,493810056	0,03274506

Table S3: List of genes significantly regulated in HUVECs by treatment with 250 nM LatB for 4h. Orange: down regulated genes, green: upregulated genes, NA: non protein coding genes.

ENSEMBL	symbol	log2 fold change	adjusted p value
ENSG00000234576	NA	-5,44594474	2,64E-05
ENSG00000278806	NA	-4,5534863	0,01021061
ENSG00000225718	NA	-3,65942707	0,0083952
ENSG00000166165	CKB	-3,64435677	4,33E-05
ENSG00000231034	NA	-3,45474852	0,02475462
ENSG00000229735	NA	-3,29860145	0,0383754
ENSG00000125378	BMP4	-1,84640849	4,19E-13
ENSG00000171388	APLN	-1,75669329	1,38E-08
ENSG00000156466	GDF6	-1,65336517	5,28E-05
ENSG00000118523	CTGF	-1,5886176	5,97E-37
ENSG00000148677	ANKRD1	-1,50746647	2,70E-27
ENSG00000078401	EDN1	-1,49884512	2,73E-28
ENSG00000142871	CYR61	-1,48015156	1,25E-31
ENSG00000176697	BDNF	-1,30977432	0,01587802
ENSG00000107984	DKK1	-1,21745678	2,50E-10
ENSG00000157168	NRG1	-1,09048674	0,00032156
ENSG00000099860	GADD45B	-1,08030402	0,00090707
ENSG00000176641	RNF152	-1,03290295	5,77E-06
ENSG00000162614	NEXN	-1,0081072	5,65E-05
ENSG00000117152	RGS4	-0,93498945	1,91E-05
ENSG00000214289	NA	-0,9112063	0,00032483
ENSG00000235926	NA	-0,83259571	0,0310095
ENSG00000074590	NUAK1	-0,82519552	0,02770423
ENSG00000235387	SPAAR	-0,81742293	0,03057397
ENSG00000275342	PRAG1	-0,81729627	0,00468475
ENSG00000178882	RFLNA	-0,80660113	0,01668169
ENSG00000144120	TMEM177	-0,74529577	0,00346445
ENSG00000065613	SLK	0,55832331	0,00321601
ENSG00000173210	ABLIM3	0,56917767	0,04387971
ENSG00000119899	SLC17A5	0,57109907	0,04076403
ENSG00000173575	CHD2	0,57492698	0,00229876
ENSG00000198873	GRK5	0,58148422	0,01506679
ENSG00000106537	TSPAN13	0,5816018	0,02516905
ENSG00000123094	RASSF8	0,5971009	0,03210881
ENSG00000138434	SSFA2	0,59797009	0,00017947
ENSG00000023287	RB1CC1	0,60298942	0,02400581
ENSG00000120875	DUSP4	0,60568084	3,11E-06
ENSG00000115993	TRAK2	0,61059133	0,03266839
ENSG00000173218	VANGL1	0,61273784	0,03479464
ENSG00000114796	KLHL24	0,61950604	0,02182263
ENSG00000169554	ZEB2	0,61984925	0,00360596
ENSG00000083223	ZCCHC6	0,62899774	0,02330445
ENSG00000073756	PTGS2	0,62948363	0,0153939

ENSG00000270106	TSNAX-DISC1	0,62954492	0,02547031
ENSG00000081189	MEF2C	0,63448471	3,93E-05
ENSG00000130766	SES2	0,63581621	0,02701907
ENSG00000153885	KCTD15	0,64004924	0,04305272
ENSG00000102802	MEDAG	0,64634328	0,03720889
ENSG00000198844	ARHGEF15	0,646879	0,00113829
ENSG00000182208	MOB2	0,64897713	0,02193628
ENSG00000128284	APOL3	0,66574637	0,00966076
ENSG00000057704	TMCC3	0,66910573	0,0240483
ENSG00000136237	RAPGEF5	0,67799611	0,04259377
ENSG00000147421	HMBOX1	0,68095517	0,00398138
ENSG00000245532	NEAT1	0,68596589	2,29E-10
ENSG00000173706	HEG1	0,68962416	7,28E-05
ENSG00000185262	UBALD2	0,6984385	4,00E-05
ENSG00000270087	NA	0,69973386	0,03080426
ENSG00000176438	SYNE3	0,70446053	0,03633968
ENSG00000182704	TSKU	0,71198787	0,00389128
ENSG00000106829	TLE4	0,71776541	0,00855571
ENSG00000139117	CPNE8	0,71785387	0,0302548
ENSG00000008294	SPAG9	0,72305503	7,15E-07
ENSG00000139112	GABARAPL1	0,73052906	1,35E-05
ENSG00000162522	KIAA1522	0,73605251	0,04171752
ENSG00000127528	KLF2	0,73714378	3,44E-05
ENSG00000148120	C9orf3	0,74059138	0,0180949
ENSG00000031081	ARHGAP31	0,75725126	0,0002442
ENSG00000174718	KIAA1551	0,76454041	0,04466995
ENSG00000134954	ETS1	0,78045459	0,00354444
ENSG00000235750	KIAA0040	0,79040678	0,0053003
ENSG00000152518	ZFP36L2	0,79056843	3,09E-07
ENSG00000111276	CDKN1B	0,79946036	0,0124363
ENSG00000139679	LPAR6	0,82781059	0,00228863
ENSG00000161940	BCL6B	0,83129616	1,71E-07
ENSG00000166510	CCDC68	0,88179459	0,00270354
ENSG00000100784	RPS6KA5	0,92198304	0,01353423
ENSG00000108840	HDAC5	0,93221944	0,01147244
ENSG00000144560	VGLL4	0,93995395	9,12E-05
ENSG00000154734	ADAMTS1	0,95255394	1,10E-07
ENSG00000157514	TSC22D3	0,9609219	0,00971846
ENSG00000106546	AHR	0,9998206	3,92E-07
ENSG00000143127	ITGA10	1,00085755	0,00029991
ENSG00000136630	HLX	1,09595384	3,49E-07
ENSG00000090776	EFNB1	1,1885352	1,09E-10
ENSG00000159167	STC1	1,36864162	1,77E-09
ENSG00000158859	ADAMTS4	1,4528086	3,95E-31
ENSG00000138061	CYP1B1	1,75541074	7,36E-12
ENSG00000143869	GDF7	3,15558201	0,03633968

Table S4: List of protein coding genes significantly regulated in HUVECs **only** by ChivoA (left) or **only** by LatB (right). Orange: down regulated genes, green: upregulated genes.

ENSEMBL	ChivoA	ENSEMBL	LatB
ENSG00000251340	MTCYBP35	ENSG00000171388	APLN
ENSG00000109846	CRYAB	ENSG00000176641	RNF152
ENSG00000165702	GFI1B	ENSG00000162614	NEXN
ENSG00000144647	POMGNT2	ENSG00000074590	NUAK1
ENSG00000146674	IGFBP3	ENSG00000275342	PRAG1
ENSG00000081692	JMJD4	ENSG00000065613	SLK
ENSG00000163739	CXCL1	ENSG00000173210	ABLIM3
ENSG00000159399	HK2	ENSG00000119899	SLC17A5
ENSG00000120217	CD274	ENSG00000198873	GRK5
ENSG00000112029	FBXO5	ENSG00000106537	TSPAN13
ENSG00000151093	OXSM	ENSG00000123094	RASSF8
ENSG00000104833	TUBB4A	ENSG00000138434	SSFA2
ENSG00000164815	ORC5	ENSG00000120875	DUSP4
ENSG00000132436	FIGNL1	ENSG00000115993	TRAK2
ENSG00000115875	SRSF7	ENSG00000173218	VANGL1
ENSG00000183741	CBX6	ENSG00000114796	KLHL24
ENSG00000092098	RNF31	ENSG00000169554	ZEB2
ENSG00000127824	TUBA4A	ENSG00000083223	ZCCHC6
ENSG00000179431	FJX1	ENSG00000270106	TSNAX-DISC1
ENSG00000182253	SYNM	ENSG00000081189	MEF2C
ENSG00000146834	MEPCE	ENSG00000130766	SESN2
ENSG00000122971	ACADS	ENSG00000153885	KCTD15
ENSG00000189369	GSPT2	ENSG00000102802	MEDAG
ENSG00000155438	NIFK	ENSG00000198844	ARHGEF15
ENSG00000137267	TUBB2A	ENSG00000182208	MOB2
ENSG00000099260	PALMD	ENSG00000128284	APOL3
ENSG00000119408	NEK6	ENSG00000057704	TMCC3
ENSG00000188229	TUBB4B	ENSG00000136237	RAPGEF5
ENSG00000137285	TUBB2B	ENSG00000147421	HMBOX1
ENSG00000130340	SNX9	ENSG00000173706	HEG1
ENSG00000275023	MLLT6	ENSG00000185262	UBALD2
ENSG00000130513	GDF15	ENSG00000176438	SYNE3
ENSG00000088826	SMOX	ENSG00000182704	TSKU
ENSG00000257242	LINC01619	ENSG00000106829	TLE4
ENSG00000215788	TNFRSF25	ENSG00000139117	CPNE8
ENSG00000188559	RALGAPA2	ENSG00000008294	SPAG9
ENSG00000167106	FAM102A	ENSG00000139112	GABARAPL1
ENSG00000168916	ZNF608	ENSG00000235750	KIAA0040
ENSG00000101445	PPP1R16B	ENSG00000111276	CDKN1B
ENSG00000119138	KLF9	ENSG00000139679	LPAR6
ENSG00000109787	KLF3	ENSG00000108840	HDAC5
ENSG00000100916	BRMS1L	ENSG00000106546	AHR
ENSG00000164463	CREBRF	ENSG00000143127	ITGA10
ENSG00000183337	BCOR	ENSG00000090776	EFNB1
ENSG00000136826	KLF4	ENSG00000143869	GDF7
ENSG00000154240	CEP112		
ENSG00000266010	GATA6-AS1		
ENSG00000134323	MYCN		