A 2D-DIGE-based proteomic analysis reveals differences in the platelet releasate composition when comparing thrombin and collagen stimulations

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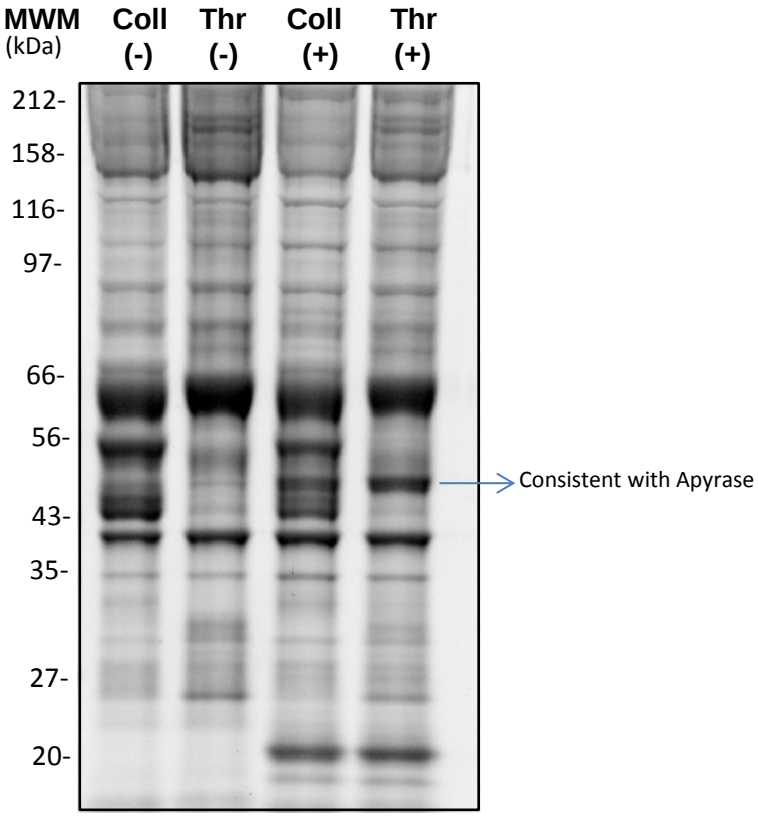
Supplementary Figure 1



Supplementary Figure 1. Releasate profile comparison following platelet activation with thrombin and collagen in presence and absence of inhibitors of secondary mediators and integrilin (αIIbβ3 inhibitor). Platelets were activated for 3 min with 0.75 U/mL thrombin or 30 µg/mL collagen. When indicated, platelets were pre-incubated for 5 min with the following inhibitors: 2 U/mL apyrase, 10 µM indomethacin, and 9 µM integrilin. Releasates were obtained as indicated in the Methods section. Secreted proteins were separated by 1D-SDS-PAGE in 4-12% Nu-PAGE Bis-Tris gels (Invitrogen). Ten micrograms of protein were loaded per lane. The gel was stained with Sypro Ruby fluorescent dye (Lonza). MWM: molecular weight marker; Coll(-): collagen without inhibitors; Thr(-): thrombin without inhibitors; Coll(+): collagen with inhibitors; Thr(+): thrombin with inhibitors

Supplementary Table 1. Additional MS data.

Spot	N† / %‡	Mass score	Peptides identified by MS		Identified protein	UniProt Code	MW (estimated)/ MW (theo)	pI (estimated/ pI (theo)	Fold change
			m/z	Sequence					
477	--	--	--	--	Not identified	--	--	--	4.6
481	--	--	--	--	Not identified	--	--	--	2.3
672	6/5	167.0	729.459 845.478 1337.725 1419.724 1465.816 1582.859	IQKNVK TILDDLR FYNQVSTPLLR VQFELHYQEVK KFYNQVSTPLLR IQPSGGTNINEALLR	Inter-alpha-trypsin inhibitor heavy chain H2[aa]	ITIH2_HUMAN	78.953/ 106.463	5.89/6.40	3.6
680	4/14	79.0	1336.634 1356.723 1859.976 2244.144	FYEEVHDLER YAVLYQPLFDK LDGLVETPTGYIESLPR QLTVQMMQNPQILAALQER	Nucleosome assembly protein 1-like 1[aa]	NP1L1_HUMAN	77.161/45.374	4.51/4.63	2.3
687	4/13.1	170.3	554.3190 407.1541 424.2111 699.6625	EHAVEGDCDFQLLK FSVVYAK CNLLAEK HTFMGVVSLGSPSGEVSHPR	Alpha-2-HS-glycoprotein	FETUA_HUMAN	73.578/39.324	4.59/5.43	2.1
688	--	--	--	--	Not identified	--	--	--	2.5
716	--	--	--	--	Not identified	--	--	--	-2.3
775	10/29	169.0	1077.529 1130.598 1142.611 1159.627 1334.645 1415.749 1631.829 1695.842 2284.175 2312.136	IREEYPDR FPGQLNADLR YLTVACIFR LAVNMVPFPR ISVYYNEAYGR EVDQQLLSVQTR AVLVDLEPGTMDSIR ALSVAELTQQMFDAR GLSMAATFIGNNTAIQEIFNR LHFFMPGFAPLTAQGSQQYR	Tubulin beta-1 chain[aa]	TBB1_HUMAN	52.373/50.326	5.24/5.05	-4.6
	11/32	101.0	759.448 1034.533 1117.536 1150.518 1293.753 1491.749 1513.730 1682.960 2011.958 2207.092 2536.255	IIPFNR VGPEADKYR VELEDWNGR TSTADYAMFK QSGLYFIKPLK YEASILTHDSSIR YLQEIYNSNNQK IHLISTQSAIPYALR CHAGHLNGVYYQGGTYSK EGFGHLSPTGTTEFWLGNEK AIQLTYNPDESSKPNMIDAATLK	Fibrinogen gamma chain[aa]	FIBG_HUMAN	52.373/51.511	5.24/5.37	
776	14/39	364.0	944.518	GASALQLER	Tubulin beta-1 chain[aa]	TBB1_HUMAN	52.373/50.326	5.28/5.05	-2.5

			1077.534 1130.600 1142.605 1159.627 1287.716 1334.645 1415.753 1631.838 1695.840 2028.032 2284.168 2312.123 2336.995	IREEYPDR FPGQLNADLR YLTVACIFR LAVNMVPFPR KLAVNMVPFPR ISVYYNEAYGR EVDQQLLSVQTR AVLVDLEPGTMDSIR ALSVAELTQQMFDAR GHYTEGAELIENVLEVVR GLSMAATFIGNNTAIQEIFNR LHFFMPGFAPLTAQGSQQYR AVLEEDEEVTEEAEMEPEDK					
778	17/54	504.0	808.356 944.518 1077.535 1130.601 1142.606 1159.628 1198.562 1334.642 1415.754 1631.836 1695.839 2028.041 2284.165 2296.151 2336.991 2707.389 3808.836	EEYPDR GASALQLER IREEYPDR FPGQLNADLR YLTVACIFR LAVNMVPFPR VSEHFSAMFK ISVYYNEAYGR EVDQQLLSVQTR AVLVDLEPGTMDSIR ALSVAELTQQMFDAR GHYTEGAELIENVLEVVR GLSMAATFIGNNTAIQEIFNR LHFFMPGFAPLTAQGSQQYR AVLEEDEEVTEEAEMEPEDK LTTPTYGDLNHLVSLTMSGITTSLR HESESCDCLQGFQIVHSLGGGTGSGMGTLLMN KIR	Tubulin beta-1 chain[a]	TBB1_HUMAN	52.075/50.326	5.20/5.05	-2.4
800	5/10	53.0	960.563 1358.639 1467.835 1910.929 2045.092	FQNALLVR AVMDDFAAFVEK RHPDYSVVLLLR RPCFSALEVDETYVPK VFDEFKPLVEEPQNLIK	Serum albumin[aa]	ALBU_HUMAN	47.395/69.366	6.01/5.92	-5.4
815	9/33	313.0	795.468 976.452 1132.531 1516.714 1639.797 1790.898 1954.056 1962.914 2359.144	IIAPPER AGFAGDDAPR GYSFTTTAER QEYDESGPSIVHR LDLAGRDLTDYLMK SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK YPIEHGIVTNWDDMEK KDLYANTVLSGGTTMYPGIADR	Actin cytoplasmic 2[aa]	ACTG_HUMAN	46.514/41.792	5.25/5.31	-2.7

	5/7.8	175.0	976.452 1516.714 1790.898 2280.143 2720.284	AGFAGDDAPR QEYDESGPSIVHR SYELPDGQVITIGNER MVVEVDSMPAASSVKKPFGLR DLYTNTVLSGGTTMYPGMAHRMQK	POTE ankyrin domain family member E[aa]	POTEE_HUMAN	46.514/121.363	5.25/5.83	
	3/13.3	174.0	1189.629 1516.714 1790.898	EITALAPSIMK QEYDESGPSIVHR SYELPDGQVITIGNER	Putative beta-actin-like protein 3[aa]	ACTBM_HUMAN	46.514/42.016	5.25/5.91	
819	7/15	103.0	960.561 1149.574 1358.640 1467.836 1910.933 2045.095 2260.034	FQNALLVR DAHKSEVAHR AVMDDFAAFVEK RHPDYSVVLLLR RPCFSALEVDETYVPK VFDEFKPLVEEPQNLIK EFNAETFTFHADICTLSEK	Serum albumin[aa]	ALBU_HUMAN	46.394/69.366	5.77/5.92	-11.5
855	--	--	--	--	Not identified	--	--	--	-3.9
899	--	--	--	--	Not identified	--	--	--	-2.5
956	--	--	--	--	Not identified	--	--	--	-3.7
961	3/7	164.0	779.403 1288.643 1873.992	ALQEYR ELDESLQVAER LFDSDPITVTVPVEVSR	Clusterin[aa]	CLUS_HUMAN	40.827/52.494	4.70/5.88	-3.2
962	--	--	--	--	Not identified	--	--	--	2.6
973	3/6	140.0	732.402 1075.577 1393.699	SGSGLVGR RPHFFFPK ASSIIDELFQDR	Clusterin[aa]	CLUS_HUMAN	40.547/52.494	4.75/5.88	-4.2
1086	--	--	--	--	Not identified	--	--	--	3.4
1103	--	--	--	--	Not identified	--	--	--	-9.0
1111	8/33.9	480.1	733.6163 584.7448 631.2872 818.3149 637.1970 680.8106 572.7898 565.2346	VGVKPVGSDPDFQPELSGAGSR IAPAFSSMSNK VRIDQYQGADAVGLEEK IDQYQGADAVGLEEK QHLENDPGSNEDTDIPK GYMDLMPFINK FQGPDNGQGPK SMDFEEAER	Thioredoxin-like protein 1	TXNL1_HUMAN	35.301/32.251	4.99/4.84	-4.7
	2/7.3	92.2	536.3023 771.9748	EIQNAVNGVK VTTVASHTSDSDVPSGVTEVVVK	Clusterin	CLUS_HUMAN	35.301/52.494	4.99/5.88	
1199	1/4.0	65.0	559.6930	SGTDVDAANLR	Caspase-3	CASP3_HUMAN	31.937/31.607	6.43/6.09	-2.2
1203	7/7	291.0	1299.698 1330.694 1376.711 1417.748 1759.839 2083.019 2129.066	EDTSPAVLGLAAR LANPLHFYEAR IETSSLDGENRR ESYNVQLQLPAR SEGPYFSLTSTEQSVK SHLYGITAVYPYCPTGRK FSNLYVGTNGIISTQDFPR	Nidogen-2[aa]	NID2_HUMAN	31.657/151.253	4.88/5.09	2.5

1226	8/8	355.0	989.616 1030.505 1062.566 1207.637 1394.738 1616.819 1864.908 2195.072	GFLLLASLR GPDPSSPAFR FQDLVDAVR SITLFVQEDR FVFGTTPEDILR GGVNDNFQGVLQNVR MENAELDVPIQSVFTR IPESGGDNSVFDIFELTGAAR	Thrombospondin-1[aa]	TSP1_HUMAN	30.736/129.382	5.60/4.71	-2.0
1244	5/14.8	284.9	846.0040 497.8626 757.2810 647.3497 440.6205	AIQLTYNPDESSKPNMIDAATLK YEASILTHDSSIR YLQEIYNSNNQK QSGLYFIKPLK LDGSVDFK	Fibrinogen gamma chain	FIBG_HUMAN	29.694/51.511	5.57/5.37	-4.3
	1/1.1	74.0	716.8663	LVEENALAPDFSK	Multimerin-1	MMRN1_HUMAN	29.694/138.110	5.57/8.15	
1263	--	--	--	--	Not identified	--	--	--	2.7
1269	--	--	--	--	Not identified	--	--	--	2.1
1271	--	--	--	--	Not identified	--	--	--	5.2
1274	2/10.1	123.9	617.2464 497.2080	GFQALGDAADIR SEEFLIAGK	Metalloproteinase inhibitor 1	TIMP1_HUMAN	28.373/23.170	6.01/8.46	2.4
	1/6.6	75.2	851.8697	DDVGGAQDVGMLGILVK	Haloacid dehalogenase-like hydrolase domain-containing protein 2	HDHD2_HUMAN	28.373/28.535	6.01/5.84	
1275	7/13	57.0	927.495 960.563 1149.610 1467.839 1742.849 1910.921 2045.053	YLYEIAR FQNALLVR LVNEVTEFAK RHPDYSVVLLLR HPYFYAPELLFFAK RPCFSALEVDETYVPK VFDEFKPLVEEPQNLIK	Serum albumin[aa]	ALBU_HUMAN	28.333/69.366	5.71/5.92	3.2
1278	--	--	--	--	Not identified	--	--	--	2.4
1292	--	--	--	--	Not identified	--	--	--	3.0
1301	2/32.1	111.2	712.5689 752.3000	VDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSK	Ig kappa chain C region	IGKC_HUMAN	27.532/11.608	5.42/5.58	2.1
	1/17.9	57.3	993.4045	AAPSVTLFPPSSEELQANK	Ig lambda-2 chain C regions	LAC2_HUMAN	27.532/11.293	5.42/6.91	
1309	2/7.5	76.5	845.9940 647.3155	AIQLTYNPDESSKPNMIDAATLK QSGLYFIKPLK	Fibrinogen gamma chain	FIBG_HUMAN	27.051/51.511	5.98/5.37	-3.5
	1/1.1	81.3	716.7845	LVEENALAPDFSK	Multimerin-1	MMRN1_HUMAN	27.051/138.110	5.98/8.15	
	1/3.6	40.7	428.6747	LGAETLPR	Cardiotrophin-like cytokine factor 1	CLCF1_HUMAN	27.051/24.086	5.98/8.91	
1348	6/27.9	398.8	601.7407 959.3426 485.5411 809.2749 631.1764 876.3069	SIQEIQELDK SIQEIQELDKDDESLR QSFVLKEGVEYR IDKTDYMVGSYGPR TDYMVGSYGPR AEEYEFLTPVEEAPK	Rho GDP-dissociation inhibitor 1	GDIR1_HUMAN	25.009/23.207	5.03/5.01	8.9

1373	5/25.9	397.8	970.2622 854.8027 656.2696 716.6494 428.2652	APEPHVEEDDDDELDSK ELQEMDKDDESLIK TLLGDGPVVTDPK TLLGDGPVVTDPKAPNVVVTR APNVVVTR	Rho GDP-dissociation inhibitor 2	GDIR2_HUMAN	24.591/22.988	5.23/5.08	3.1
1378	2/13.0	199.6	542.3423 558.2401 555.7220	TTTGSYIANR LAAIAESGVER QVLLGDQIPK	Proteasome subunit beta type-6	PSB6_HUMAN	24.519/25.357	4.94/4.80	8.3
1393	3/14.3	145.1	517.7174 436.2565 615.7708	IPTTFENGR VLNQELR NILTSNNIDVK	Apolipoprotein D	APOD_HUMAN	24.290/21.275	5.05/5.06	3.6
	1/5.1	60.2	771.9686	VTTVASHTSDSDVPSGVTEVVVK	Clusterin	CLUS_HUMAN	24.290/52.494	5.05/5.88	
1397	4/15.7	296.7	908.2943 700.7830 806.8016 608.2316	DSGRDYVSQFEGSALGK DYVSQFEGSALGK LLDNWDSVTSTFSK ATEHLSTLSEK	Apolipoprotein A-I	APOA1_HUMAN	24.218/30.777	5.42/5.56	-3.9
	1/5.2	47.3	669.3233	PPYTVVYFPVR	Glutathione S-transferase P	GSTP1_HUMAN	24.218/23.355	5.42/5.43	
1413	9/31.8	555.2	908.3457 700.7926 806.8114 626.7458 734.3038 437.1680 524.1869 651.2347 608.2018	DSGRDYVSQFEGSALGK DYVSQFEGSALGK LLDNWDSVTSTFSK VQPYLDDFQK VEPLRAELQEGAR AELQEGAR LSPLGEEMR THLAPYSDELR ATEHLSTLSEK	Apolipoprotein A-I	APOA1_HUMAN	23.976/30.777	5.30/5.56	3.8
1416	--	--	--	--	Not identified	--	--	--	5.9
1438	2/10	58.0	1169.673 1313.588	FLVGPDGVPLR DYTQMNELQR	Glutathione peroxidase 1[aa]	GPX1_HUMAN	23.627/22.088	5.72/6.15	3.4
1461	--	--	--	--	Not identified	--	--	--	3.2
1508	7/49	75.0	820.430 1218.627 1231.686 1279.603 1415.605 1694.818 3346.679	GPAYGLSR NVIGLQMGTNR TLMNLGGLAVAR NFSDNQLQEGK GASQAGMTGYGMPR QMEQISQFLQAAER ENFQNWLKDGTVLCELINALYPEGQAPVK	Transgelin-2[aa]	TAGL2_HUMAN	22.288/22.391	5.63/8.41	2.6
1516	6/32.5	330.4	657.1674 530.2144 669.0071 929.8311 585.3046 442.5360	DYTQMNELQR NEEILNSLK YVRPGGGFEPNFMLFEK EALPAPSDDATALMTDPK FLVGPDGVPLR FLVGPDGVPLRR	Glutathione peroxidase 1	GPX1_HUMAN	22.192/22.088	5.73/6.15	2.9
1530	--	--	--	--	Not identified	--	--	--	3.3
1540	--	--	--	--	Not identified	--	--	--	-3.9

1553	--	--	--	--	Not identified	--	--	--	6.0
1558	--	--	--	--	Not identified	--	--	--	10.2
1559	2/1	114.0	1894.914 2293.081	AADIEQQAVFAVFDENK LELQGCEVNGCSTPLGMENGK	Coagulation factor V[aa]	FA5_HUMAN	21.348/251.703	4.69/5.68	10.9
1560	4/28	238.0	1246.587 1253.558 1449.585 2106.977	GNFNYVEFTR EAFNMIDQNR FTDEEVDEMYR ATSNVFAMFDQSQIQEFK	Myosin regulatory light polypeptide 9[aa]	MYL9_HUMAN	21.336/19.827	4.72/4.78	8.5
1585	1/6.0	59.8	493.2444	LVVLGSGGVGK.	Ras-related protein Rap-1A	RAP1A_HUMAN	20.853/20.987	4.94/6.93	3.0
1595	--	--	--	--	Not identified	--	--	--	4.8
1647	--	--	--	--	Not identified	--	--	--	4.2
1650	--	--	--	--	Not identified	--	--	--	9.1
1654	--	--	--	--	Not identified	--	--	--	6.2
1657	--	--	--	--	Not identified	--	--	--	7.4
1658	--	--	--	--	Not identified	--	--	--	12.9
1663	--	--	--	--	Not identified	--	--	--	3.4
1669	--	--	--	--	Not identified	--	--	--	4.7
1672	1/4.0	85.2	801.7602	ADFSGMSQTDLSLSK	Serpin B6[aa]	SPB6_HUMAN	19.093/42.621	4.48/5.0	4.3
1673	--	--	--	--	Not identified	--	--	--	16.6
1674	--	--	--	--	Not identified	--	--	--	18.1
1684	--	--	--	--	Not identified	--	--	--	17.7
1727	--	--	--	--	Not identified	--	--	--	-4.1
1730	1/10.8	56.4	528.7684	VYSTSVTGSR	SH3 domain-binding glutamic acid-rich-like protein 3[aa]	SH3L3_HUMAN	17.374/10.437	4.55/4.7	4.3
1738	--	--	--	--	Not identified	--	--	--	5.6
1739	--	--	--	--	Not identified	--	--	--	4.1
1753	--	--	--	--	Not identified	--	--	--	2.2
1800	--	--	--	--	Not identified	--	--	--	-2.8
1818	14/6	221.0	705.392 767.403 845.482 977.511 1015.597 1097.493 1169.600 1312.656 1406.679 1520.772 1700.733 1738.872 2408.111 2864.336	LECRK STSVFAR TNTGLALR YPPGTSLSR AVSPLPYLR AFVVDMMER LSGEAYGFVAR SFSIIGDFQNGK GLYLETEAGYYK IDGSGNFQVLLSDR LSYGEDLQMDWDGR YFTFSGICQYLLAR CLPSACEVVTGSPRGDSQSSWK VPLDSSPATCHNNIMKQTMVDSSCR	von Willebrand factor[aa]	VWF_HUMAN	148.539/309.264	4.52/5.29	2.1
	4/4	103.0	845.482 1337.724	TILDDLR FYNQVSTPLLR	Inter-alpha-trypsin inhibitor heavy chain H2[aa]	ITIH2_HUMAN	148.539/106.463	4.52/6.40	

			1419.719 1582.854	VQFELHYQEVK IQPSGGTNINEALLR					
1825	--	--	--	--	Not identified	--	--	--	-8.6
1838	--	--	--	--	Not identified	--	--	--	5.4
1839	3/4	63.0	956.500 1018.466 1749.945	VYFAGFPR FSAEFDFR SQDILLSVENTVIYR	Vitamin K-dependent protein S[aa]	PROS_HUMAN	128.828/75.122	4.80/5.48	2.0
1842	5/5	141.0	792.433 845.478 1419.723 1465.799 1582.859	ALYAQAR TILDDLR VQFELHYQEVK KFYNQVSTPLLR IQPSGGTNINEALLR	Inter-alpha-trypsin inhibitor heavy chain H2[aa]	ITIH2_HUMAN	155.109/106.463	4.61/6.40	4.5
1843	15/21	163.0	824.423 829.431 904.463 963.460 987.501 1099.587 1144.543 1228.617 1266.672 1374.651 1462.596 1755.846 1914.860 1996.901 2022.151	HFEHVR FLHQER HVFNMLK VEAMLNDR SQVMTHLR AVIQHFQEK WYFDVTEGK QQLVETHMAR THPHFVIPYR VESLEQEAANER CAPFFYGGCGGNR ISYGNDALMPSLTETK YLETPGDENEHAHFQK EQNYSDDVLANMISEPR LALENYITALQAVPPRPR	Amyloid beta A4 protein[aa]	A4_HUMAN	149.136/83.943	4.49/4.73	2.4
	5/5	144.0	732.398 845.481 1337.721 1419.725 1582.852	TQVADAK TILDDLR FYNQVSTPLLR VQFELHYQEVK IQPSGGTNINEALLR	Inter-alpha-trypsin inhibitor heavy chain H2[aa]	ITIH2_HUMAN	149.136/106.463	4.49/6.40	
1860	5/15.0	296.1	845.9940 497.8477 757.2816 647.3559 559.2233	AIQLTYNPDESSKPNMIDAATLK YEASILTHDSSIR YLQEIYNSNNQK QSGLYFIKPLK VELEDWNGR	Fibrinogen gamma chain	FIBG_HUMAN	47.275/51.511	5.78/5.37	-3.7
	4/10.8	222.3	882.4243 514.7682 912.8290 485.6713	QIEINTISASFGGLASR AIENELLAR EGGGNNLYGEEMVQALK ASYILMEK	Glutathione synthetase	GSHB_HUMAN	47.275/52.384	5.78/5.67	
1866	2/24.5	124.2	683.8579 817.9774	GSPAINVAVHVFR ALGISPFHEHAEVVFTANDSGPR	Transthyretin[aa]	TTHY_HUMAN	18.960/15.887	4.94/5.49	2.6
1867	--	--	--	--	Not identified	--	--	--	2.9
1869	6/5	152.0	732.395	TQVADAK	Inter-alpha-trypsin inhibitor heavy chain H2[aa]	ITIH2_HUMAN	77.460/106.463	5.99/6.40	4.7

			845.480 1337.718 1419.719 1465.803 1582.852	TILDDLR FYNQVSTPLLR VQFELHYQEVK KFYNQVSTPLLR IQPSGGTNINEALLR					
1874	3/20.6	176.7	639.9150 631.1940 876.3501	SIQEIQELDKDDESLR TDYMVGSYGPR AEEYEFLTPVEEAPK	Rho GDP-dissociation inhibitor 1	GDIR1_HUMAN	25.370/23.207	4.92/5.01	6.7
1875	--	--	--	--	Not identified	--	--	--	-6.3
1876	--	--	--	--	Not identified	--	--	--	-3.1

Identifications were by ESI-TRAP unless stated otherwise. Proteins identified by [a]MALDI-MS or [aa]MALDI-MS/MS. † Number of matched peptides. ‡ % coverage of full length protein by tryptic peptides. All differential proteins have a p value lower than 0.05. A positive fold change indicates that the protein feature is up-regulated in the releasate of thrombin-stimulated platelets, whereas a negative fold change indicates that the protein feature is up-regulated in the platelet-collagen releasate.

Supplementary Methods

MS analysis

For MALDI analysis, dried peptides were dissolved in 4 μL of 0.5% formic acid. Equal volumes (0.5 μL) of peptide and matrix solution (3 mg alpha-cyano-4-hydroxycinnamic acid (α-CHCA) dissolved in 1 mL of 50% acetonitrile in 0.1% trifluoroacetic acid) were deposited using the thin layer method, onto a 384 Opti-TOF 123x81 mm MALDI plate (Applied Biosystems) and allowed to dry at room temperature. Mass spectrometric data were obtained in an automated analysis loop using a 4800 MALDI-TOF/ TOF analyzer (Applied Biosystems). Spectra were acquired in the reflector positive-ion mode with a Nd:YAG, 355nm wavelength laser, at 200 Hz laser frequency, and 1000 to 2000 individual spectra were averaged. The experiments were acquired uniform with fixed laser intensity. For MS/MS 1kV analysis mode, precursors were accelerated to 8 kV in source 1, selected with a relative resolution of 200 (FWHM) and metastable suppression. Fragment ions generated by collision with air in a CID chamber were further accelerated by 15 kV in source 2. Automated analysis of mass data was performed using the 4000 Series Explorer Software version 3.5.3 (Applied Biosystems). Internal calibration of MALDI-TOF mass spectra was performed using two trypsin autolysis ions with m/z = 842.510 and m/z = 2211.105. For MALDI-MS/MS, calibrations were performed with fragment ion spectra obtained for Glub-fibrinopeptide (4700 Cal Mix, Applied Biosystems). MALDI-MS and MS/MS data were combined through the GPS Explorer Software Version 3.6 to search a non-redundant protein database (Swissprot 2012_10) using the Mascot software version 2.2 (Matrix Science), with 50 ppm precursor tolerance, 0.6 Da MS/MS fragment tolerance, CAM (carbamidomethyl cystein) as fixed modification, oxidized methionine as variable modification and allowing 1 missed cleavage. MALDI-MS(/MS) spectra and database search results were manually inspected in detail using the previous software. For combined MS and MS/MS data, identifications were accepted

when Confidence Interval (C.I.%) of GPS software was 95% or higher. Since Protein Scores and Ion Scores from different searches cannot be directly compared, GPS software calculates this C.I.% in order to combine results from MS and MS/MS database searches. This coefficient value means that the probability that the observed match is a random event is lower than 5%. For PMF spectra, identifications were also accepted when (C.I.%) of GPS software was 99% or higher.

For LC-MS/MS digested peptide mixtures dissolved in 0.1% formic acid were separated in an EASY-nLC (Proxeon, Bruker Daltonik GmbH) with a reverse phase nanocolumn (Easy column SC200 C18 3μm 120A 360 μm OD/75μm ID, L=10cm) from Proxeon. Ionized peptides were analyzed in a Bruker Amazon ETD ion trap. Automated analysis of mass data was achieved by Data Analysis 4.0 and BioTools 3.2 from Bruker Daltonik GmbH. Database search was performed with the Mascot v2.4 search tool (Matrix Science, London, UK) screening SwissProt (release_56.1). Searches were restricted to human taxonomy allowing carbamidomethyl cysteine as a fixed modification and oxidized methionine as potential variable modification. Both the precursor mass tolerance and the MS/MS tolerance were set at 0.3 and 0.4 Da, respectively, allowing 1 missed tryptic cleavage site. All spectra and database results were manually inspected in detail using the above software, especially in the case of identifications based on one peptide hit. For the latter, positive identification by MS was only accepted when more than 50% y-ions were obtained for a peptide comprising at least eight amino acids long and no missed tryptic cleavage site. Positive hits corresponded to Mascot scores > 40 plus the fulfillment of the above criteria.