

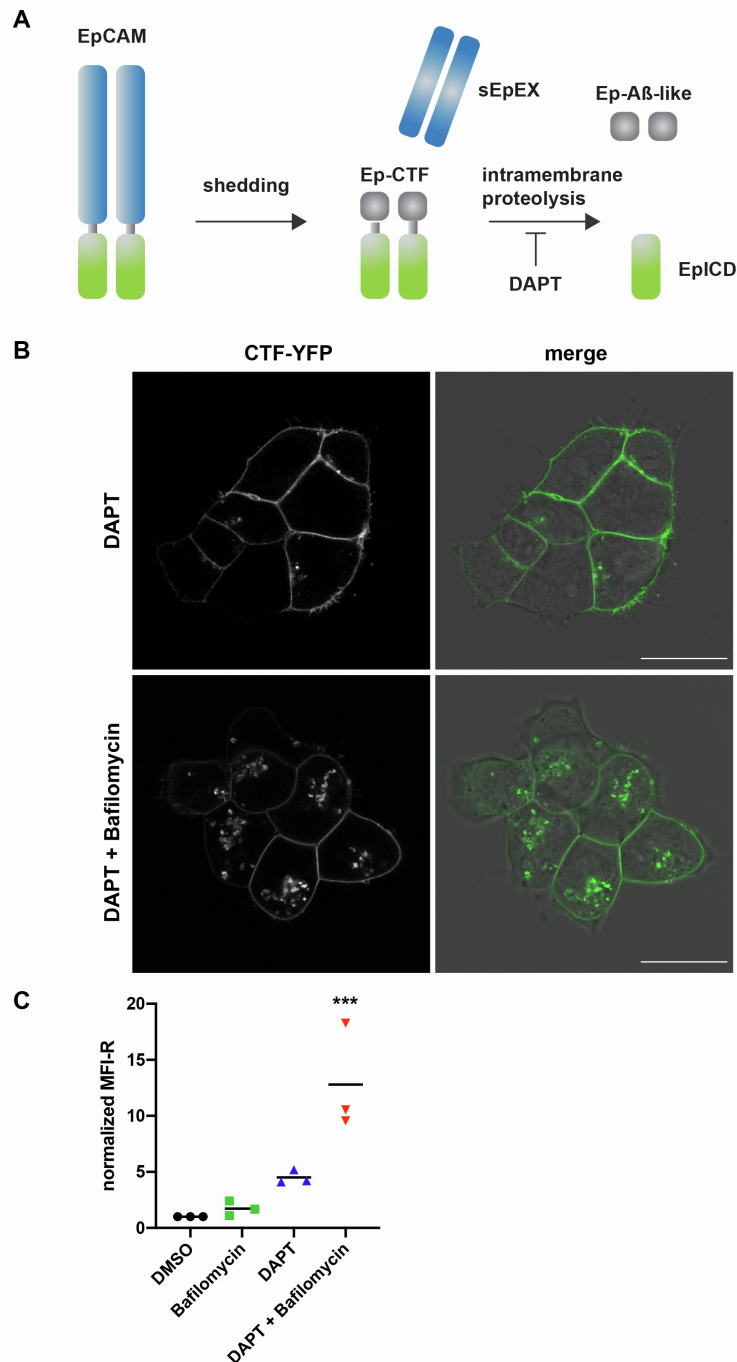
Supplemental information

Interactome analysis reveals endocytosis and membrane recycling of EpCAM during differentiation of embryonic stem cells and carcinoma cells

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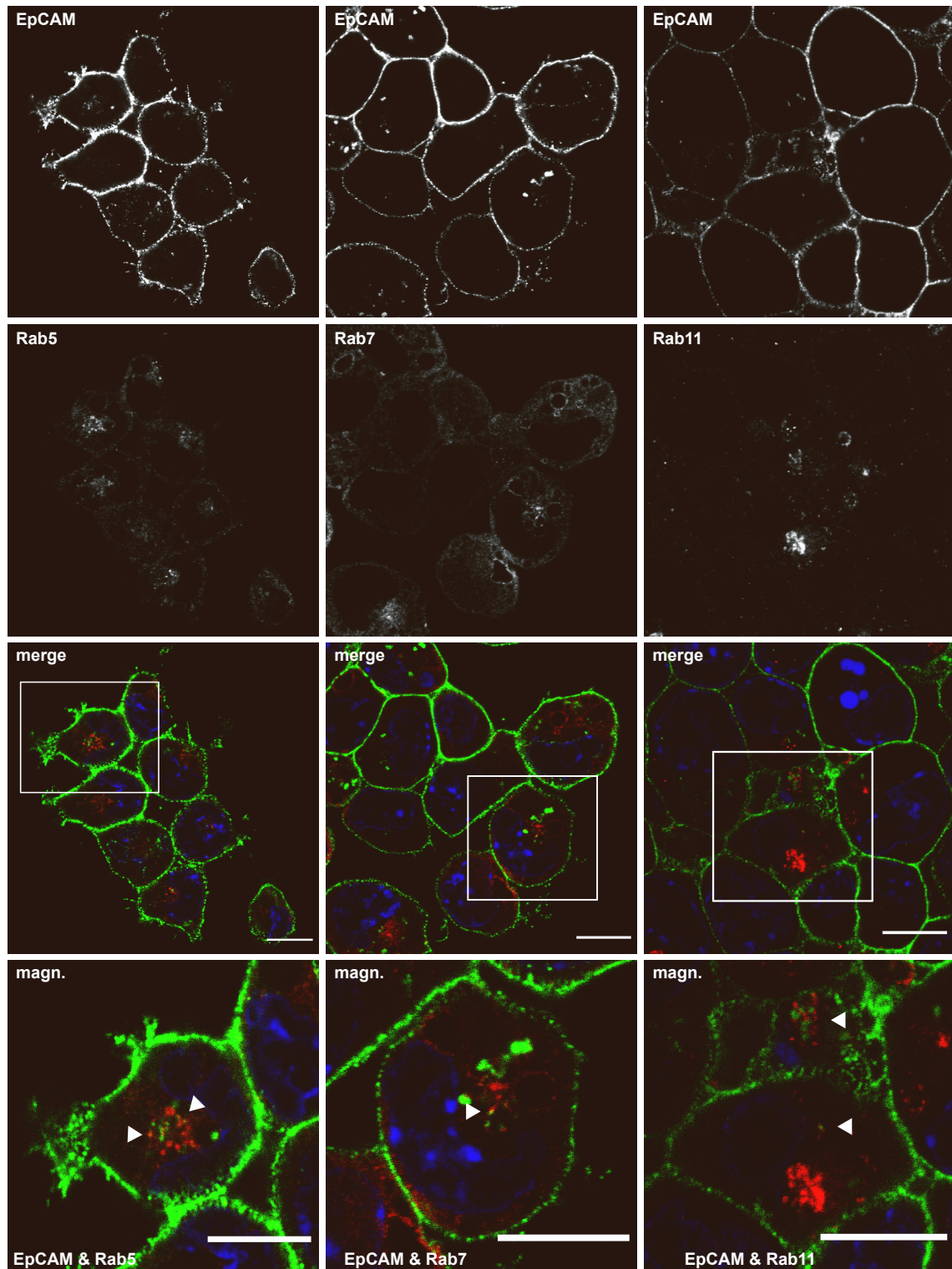
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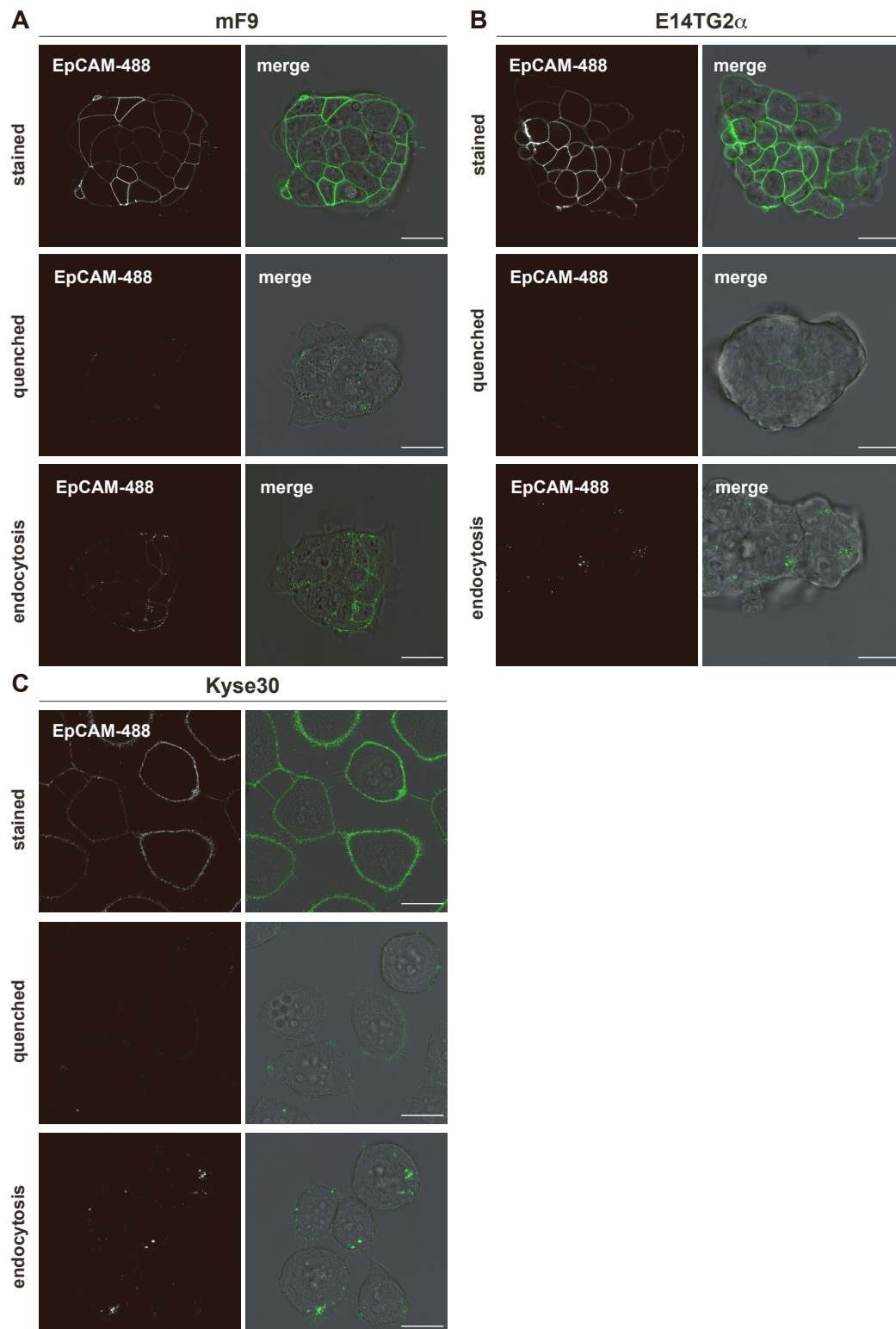
Supplementary Figure 1 (related to Figure 3): Endocytosis of the C-terminal fragment of EpCAM

(A) Schematic representation of EpCAM regulated intramembrane proteolysis. EpCAM is subject to shedding of the extracellular domain EpEX to generate the C-terminal fragment Ep-CTF, which is further cleaved through intramembrane proteolysis resulting in the formation of the intracellular domain EpICD and the extracellular Ep-Ab-like fragment. **(B)** mF9 cells stably transfected with Ep-CTF-YFP were treated with DMSO or bafilomycin A1 (10 nM) in combinations with the gamma-secretase inhibitor DAPT (10 μM). YFP fluorescence was visualized by confocal laser scanning microscopy. Shown are representative pictures with magnifications. Size scale represents 20 μm. **(C)** mF9 cells stably transfected with Ep-CTF-YFP were treated with DMSO, bafilomycin A1 (10 nM), DAPT (10 μM), or a combination of DAPT and bafilomycin A1. Mean fluorescence intensity ratios were assessed by flow cytometry. Shown are the results from 3-4 independent experiments. Mean values are indicated by a line. One-way ANOVA with DMSO as reference, p-value: *** ≤ 0.001.



Supplementary Figure 2 (related to Figure 4): EpCAM and Rab 5, Rab7, Rab11 co-staining

mF9 cells were treated with Bafilomycin A1 (10 nM) and stained with EpCAM-, Rab5-, Rab7-, and Rab11-specific antibodies as indicated in combination with Alexa-488- (EpCAM) and Alexa-594-conjugated secondary antibodies (Rab proteins). Fluorescence was visualized by confocal laser scanning microscopy. Shown are representative pictures with magnifications (magn.). Size scale represents 10 μ m in the overview and 20 μ m in magnifications.



Supplementary Figure 3 (related to Figure 4 and 5): Confocal laser scanning microscopy visualization of EpCAM endocytosis

(A-B) Wild-type mF9 (A) and E14TG2 α cells (B) were stained with Alexa-488-labelled anti-EpCAM antibody (stained). Efficiency of fluorescence quenching with an anti-Alexa-488 antibody is shown in the middle panels (quenched). Following staining with anti-EpCAM-Alexa-488 antibody, endocytosis was allowed for 30 min (see STAR Methods), cells were washed, and residual Alexa-488 fluorescence at the plasma membrane was quenched with an anti-Alexa-488 antibody (endocytosis). Alexa-488 fluorescence was visualized by confocal laser scanning microscopy for each indicated condition. Shown are representative pictures.

Supplementary Table 1 (related to Table 1): Gene ontology (GO) terms "Biological Processes" of putative mEpCAM-YFP interacting proteins. Shown are the 30 GO terms with highest enrichment levels.

GO term	GO term ID	p-value	Adj. p-value	#Genes observed	#Genes expected	#Genes total
Establishment of localization	GO:0051234	3.87E-17	0.00E+00	47	13.8	4,232
Transport	GO:0006810	6.85E-17	0.00E+00	46	13.3	4,087
Localization	GO:0051179	1.02E-14	0.00E+00	49	17.3	5,316
Vesicle-mediated transport	GO:0016192	3.88E-13	0.00E+00	23	3.6	1,098
Small GTPase-mediated signal transduction	GO:0007264	2.18E-12	0.00E+00	16	1.5	471
Organic substance transport	GO:0071702	1.24E-11	0.00E+00	30	7.6	2,341
Single-organism localization	GO:1902578	2.75E-11	0.00E+00	34	10.2	3,130
Establishment of protein localization	GO:1902578	3.52E-11	0.00E+00	26	5.8	1,798
Protein localization	GO:0045184	3.75E-11	0.00E+00	29	7.4	2,278
Cellular localization	GO:0008104	3.84E-11	0.00E+00	28	6.9	2,117
Macromolecule localization	GO:0051641	7.11E-10	0.00E+00	29	8.4	2,576
Single-organism transport	GO:0033036	7.39E-10	0.00E+00	31	9.6	2,946
Endosomal transport	GO:0044765	1.71E-09	0.00E+00	10	0.7	211
Endomembrane system organization	GO:0016197	4.62E-09	0.00E+00	13	1.5	478
Vacuole organization	GO:0010256	5.77E-09	0.00E+00	9	0.5	175
Vacuolar transport	GO:0007033	6.16E-09	0.00E+00	10	0.8	241
Protein transport	GO:0007034	6.96E-09	0.00E+00	22	5.35	1,639
Single-organism process	GO:0015031	1.43E-08	0.00E+00	66	43	13,200
Cellular protein localization	GO:0044699	1.65E-08	0.00E+00	20	4.6	1,412
Cellular macromolecule localization	GO:0034613	1.81E-08	0.00E+00	20	4.6	1,420
Intracellular signal transduction	GO:0070727	2.03E-08	0.00E+00	25	7.3	2,241
Single-organism cellular localization	GO:0035556	3.18E-08	0.00E+00	16	2.9	911
Endocytosis	GO:1902580	8.22E-08	0.00E+00	12	1.6	504
Establishment of localization in cell	GO:0006897	1.36E-07	0.00E+00	20	5.2	1,605
Regulation of vesicle-mediated transport	GO:0051649	1.57E-07	0.00E+00	11	1.4	433
Intracellular transport	GO:0060627	2.33E-07	0.00E+00	17	3.9	1,196
Exocytosis	GO:0006887	4.78E-07	0.00E+00	9	0.9	293
Receptor metabolic process	GO:0043112	7.21E-07	0.00E+00	7	0.5	154
Cell communication	GO:0007154	1.64E-06	0.00E+00	39	19.5	5,997
Golgi vesicle transport	GO:0048193	1.83E-06	0.00E+00	7	0.6	177

Supplementary Table 2 (related to Table 1): Gene ontology (GO) terms "Cellular components" of putative mEpCAM-YFP interacting proteins. Shown are the 30 GO terms with highest enrichment levels.

GO term	GO term ID	p-value	Adj. p-value	#Genes observed	#Genes expected	#Genes total
Membrane	GO:0016020	5,81E-22	0.00E+00	70	30	9205
Extracellular exosome	GO:0070062	6,17E-21	0.00E+00	42	8,5	2590
Extracellular vesicle	GO:1903561	7,47E-21	0.00E+00	42	8,5	2603
Extracellular organelle	GO:0043230	8,28E-21	0.00E+00	42	8,5	2610
Membrane-bounded vesicle	GO:0031988	1,99E-20	0.00E+00	45	10,3	3172
Endomembrane system	GO:0012505	3,71E-20	0.00E+00	46	11,1	3400
Cytoplasmic part	GO:0044444	7,19E-20	0.00E+00	60	21,5	6606
Vesicle	GO:0031982	9,22E-20	0.00E+00	46	11,3	3476
Organelle membrane	GO:0031090	9,18E-19	0.00E+00	32	4,9	1499
Whole membrane	GO:0098805	2,23E-18	0.00E+00	27	3,2	985
Bounding membrane of organelle	GO:0098588	3,40E-18	0.00E+00	26	2,9	903
Extracellular region part	GO:0044421	7,54E-17	0.00E+00	44	12,1	3704
Cytoplasm	GO:0005737	1,84E-16	0.00E+00	67	32,5	9966
Intracellular organelle part	GO:0044446	2,10E-15	0.00E+00	53	19,8	6084
Organelle part	GO:0044422	7,56E-15	0.00E+00	53	20,4	6259
Extracellular region	GO:0005576	1,44E-14	0.00E+00	44	13,9	4255
Cell part	GO:0044464	1,15E-13	0.00E+00	75	48,9	14997
Cell	GO:0005623	1,19E-13	0.00E+00	75	48,9	15005
Intracellular membrane-bounded organelle	GO:0043231	2,17E-13	0.00E+00	64	32,95	10103
Membrane-bounded organelle	GO:0043227	3,14E-13	0.00E+00	67	36,8	11285
Cytoplasmic, membrane-bounded vesicle	GO:0016023	4,19E-13	0.00E+00	20	2,5	776
Endosome	GO:0005768	7,81E-13	0.00E+00	19	2,3	703
Intracellular organelle	GO:0043229	7,85E-13	0.00E+00	66	36,1	11076
Vacuolar membrane	GO:0005774	2,42E-12	0.00E+00	14	1,05	324
Organelle	GO:0043226	3,39E-12	0.00E+00	68	39,6	12156
Vacuolar part	GO:0044437	4,45E-12	0.00E+00	14	1,1	339
Vacuole	GO:0005773	7,57E-12	0.00E+00	21	3,34	1024
Endocytic vesicle	GO:0030139	7,61E-12	0.00E+00	10	0,4	122
Intracellular part	GO:0044424	1,09E-11	0.00E+00	69	41,8	12819
intracellular	GO:0005622	1,83E-11	0.00E+00	69	42,2	12929

Supplementary Table 3 (related to Table 1): Gene ontology (GO) terms "Molecular functions" of putative mEpCAM-YFP interacting proteins. Shown are the 30 GO terms with highest enrichment levels.

GO term	GO term ID	p-value	Adj. p-value	#Genes observed	#Genes expected	#Genes total
GDP binding	GO:0019003	4,10E-19	0.00E+00	12	0,18	58
GTPase activity	GO:0003924	2,64E-14	0.00E+00	13	0,59	184
GTP binding	GO:0005525	2,65E-13	0.00E+00	15	1,10	343
Guanyl ribonucleotide binding	GO:0032561	6,24E-13	0.00E+00	15	1,17	364
Guanyl nucleotide binding	GO:0019001	6,49E-13	0.00E+00	15	1,18	365
Protein binding	GO:0005515	3,92E-10	0.00E+00	52	25,57	7903
Nucleoside-triphosphate activity	GO:0017111	4,93E-10	0.00E+00	16	2,22	687
Myosin binding	GO:0017022	9,36E-10	0.00E+00	7	0,19	60
Pyrophosphate activity	GO:0016462	1,12E-09	0.00E+00	16	2,35	727
Hydrolase activity, acting on anhydrides, In phosphorus-containing anhydrides	GO:0016818	1,16E-09	0.00E+00	16	2,35	729
Hydrolase activity, acting on anhydrides	GO:0016817	1,18E-09	0.00E+00	16	2,36	730
Enzyme binding	GO:0019899	2,00E-09	0.00E+00	24	6,02	1862
Carbohydrate derivate binding	GO:0097367	3,36E-09	0.00E+00	25	6,72	2077
Myosin V binding	GO:0031489	3,47E-09	0.00E+00	5	0,06	19
Ribonucleotide binding	GO:0032553	8,28E-08	0.00E+00	21	5,62	1737
Heterocyclic compound binding	GO:1901363	2,08E-07	0.00E+00	37	16,68	5155
Purine ribonucleoside triphosphate binding	GO:0035639	2,22E-07	0.00E+00	20	5,41	1674
Purine ribonucleoside binding	GO:0032550	2,54E-07	0.00E+00	20	5,46	1688
Ribonucleoside binding	GO:0032549	2,59E-07	0.00E+00	20	5,46	1690
Purine nucleoside binding	GO:0001883	2,61E-07	0.00E+00	20	5,47	1691
Nucleoside binding	GO:0001882	2,79E-07	0.00E+00	20	5,49	1698
Binding	GO:0005488	3,14E-07	0.00E+00	61	40,12	12400
Organic cyclic compound binding	GO:0097159	3,25E-07	0.00E+00	37	16,96	5242
Purine ribonucleotide binding	GO:0032555	3,50E-07	0.00E+00	20	5,57	1722
Purine nucleotide binding	GO:0017076	3,83E-07	0.00E+00	20	5,60	1732
Nucleotide binding	GO:0000166	7,25E-07	0.00E+00	22	6,98	2158
Nucleoside phosphate binding	GO:1901265	7,25E-07	0.00E+00	22	6,98	2158
Small molecule binding	GO:0036094	7,29E-07	0.00E+00	23	7,58	2344
Ran GTPase binding	GO:0008536	3,39E-06	0.00E+00	4	0,10	32

Glycoprotein binding	GO:0001948	4,24E-05	0.00E+00	5	0,38	119
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