a

Ras superfamily of small GTPases

RAS subfamily	RHO/RAC/CDC42 subfamily	RAB/RAN subfamily		ARF subfamily
RAS (onco) proteins: HRAS	RHOA	RABL3	RAB18	ARL15
KRAS	RHOB	IFT27	RAB33A	SAR1A
NRAS	RHOC	RASEF	RAB33B	SAR1B
RRAS	RAC1	RAB28	RAB15	ARL8A
RRAS2	RAC2	RAB10	RAB40A	ARL8B
MRAS	RAC3	RAB13	RAB40AL	ARL9
RAP1A	RHOG	RAB12	RAB40B	ARL10
RAP1B	CDC42	RAB8A	RAB40C	ARF1
RAP2A	RHOQ	RAB8B	RAB17	ARF3
RAP2B	RHOJ	RAB1A	RAB21	ARF4
RAP2C	RHOU	RAB1B	RAB5A	ARF5
RALA	RHOV	RAB35	RAB5B	ARF6
RALB	RHOBTB1	RAB26	RAB5C	TRIM23
RIT1	RHOBTB2	RAB37	RAB22A	ARL1
RIT2	RHOBTB3	RAB44	RAB31	ARL5C
ERAS	RHOH	RAB27A	RAB20	ARL5A
DIRAS1	RND1	RAB27B	RAB24	ARL5B
DIRAS2	RND2	RAB3D	RAB41	ARL4D
DIRAS3	RND3	RAB3A	RAB6A	ARL4C
RASD1	RHOD	RAB3B	RAB6B	ARL4A
RASD2	RHOF	RAB3C	RAB6C	ARL14
NKIRAS1	RHOT1	RAB42	RAB7A	ARL11
NKIRAS2	RHOT2	RAB4A	RAB7B	ARL2
REM1		RAB4B	RAB9A	ARL3
REM2		RAB14	RAB9B	ARL6
RRAD		RAB2A	RAN	ARFRP1
GEM		RAB2B	RABL2A	ARL13A
RERG		RAB25	RABL2B	ARL13B
RERGL		RAB11A	RAB29	ARL16
RASL10A		RAB11B	RAB32	ARL17A
RASL10B		RAB39A	RAB38	ARL17B
RASL11A		RAB39B	RAB23	
RASL11B		RAB19	RAB34	
RASL12		RAB30	RAB36	
RHEB		RAB43	IFT22	
RHEBL1				

b

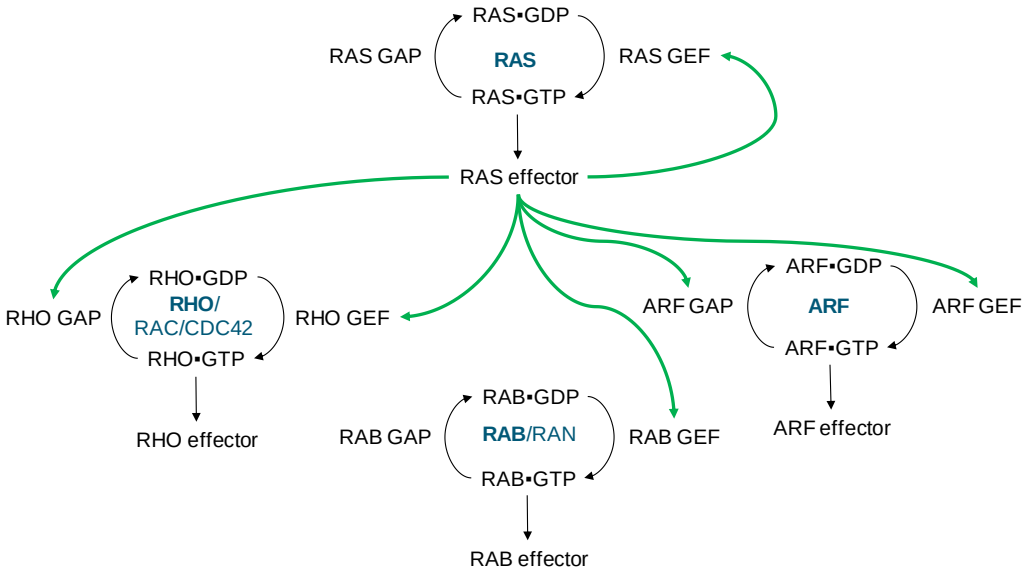

Figure S1. Members of the Ras superfamily and crosstalk. (**a**) Members of the four subfamilies of the human RAS superfamily based on domain predictions using the Pfam (https://pfam.xfam.org/) and SMART (http://smart.embl-heidelberg.de/) databases, and manual curation (gene IDs). (**b**). Crosstalk between the RAS superfamily members through their GEFs, GAPs, and effectors. In particular, several Ras effectors contain GEF or GAP domain for other Ras superfamily members, thus suggesting the Ras subfamily upstream of other Ras superfamily small GTPases.

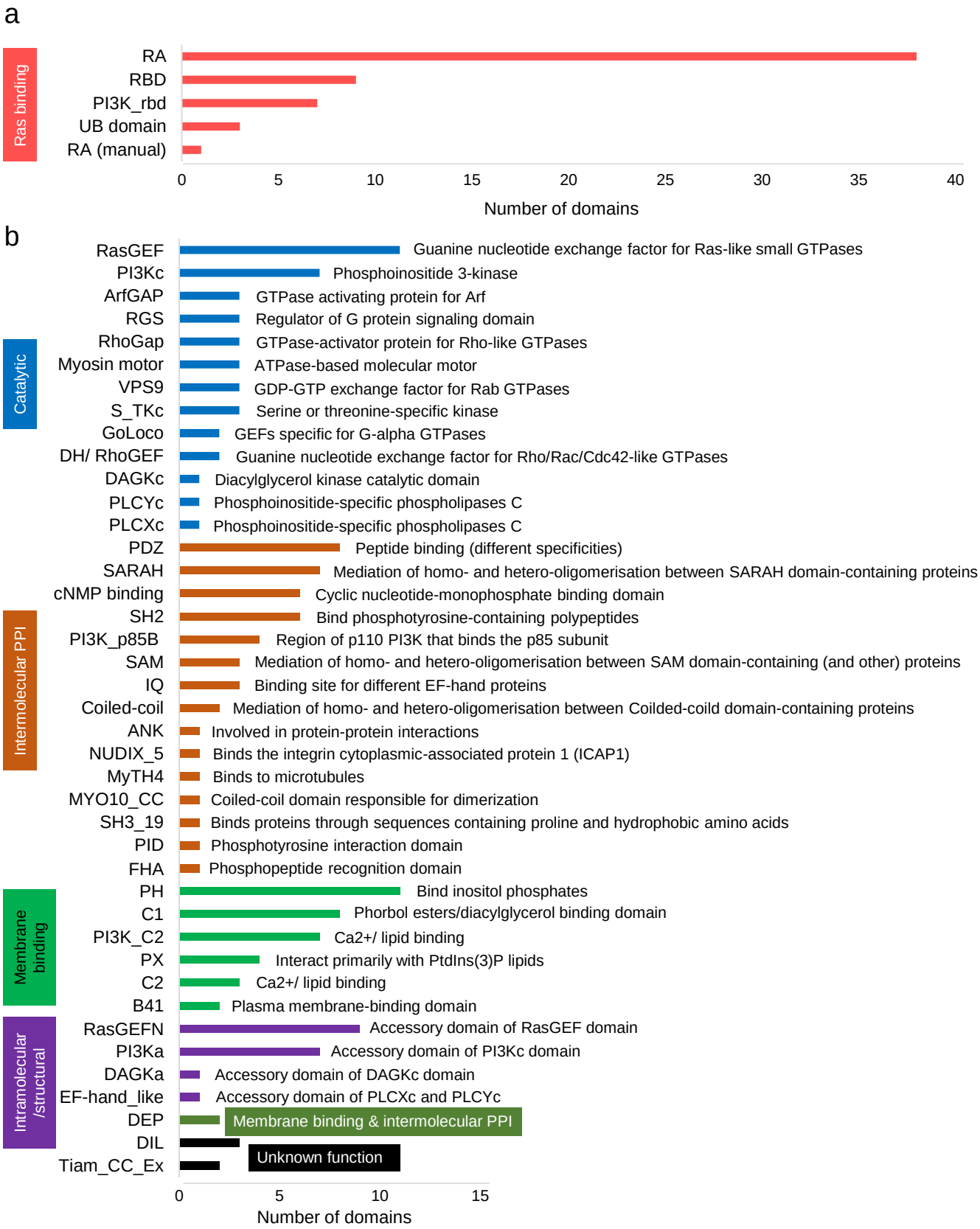

Figure S2. Classification of domains in Ras effectors and their distribution. (**a**) Distribution of Ras binding domains classified into RA, RBD, PI3K_rbd, and UB domains. (**b**) Distribution of additional domains present in the 56 Ras-effectors, and classification into catalytic domains (blue), intermolecular protein-protein interaction (PPI)-mediated domains (orange), membrane-binding domains (green), and intramolecular/structural domains (purple). The DEP domain (olive green) is involved in both, membrane binding and intermolecular PPI, and the DIL and Tiam_CC_Ex domains do not have a known function.

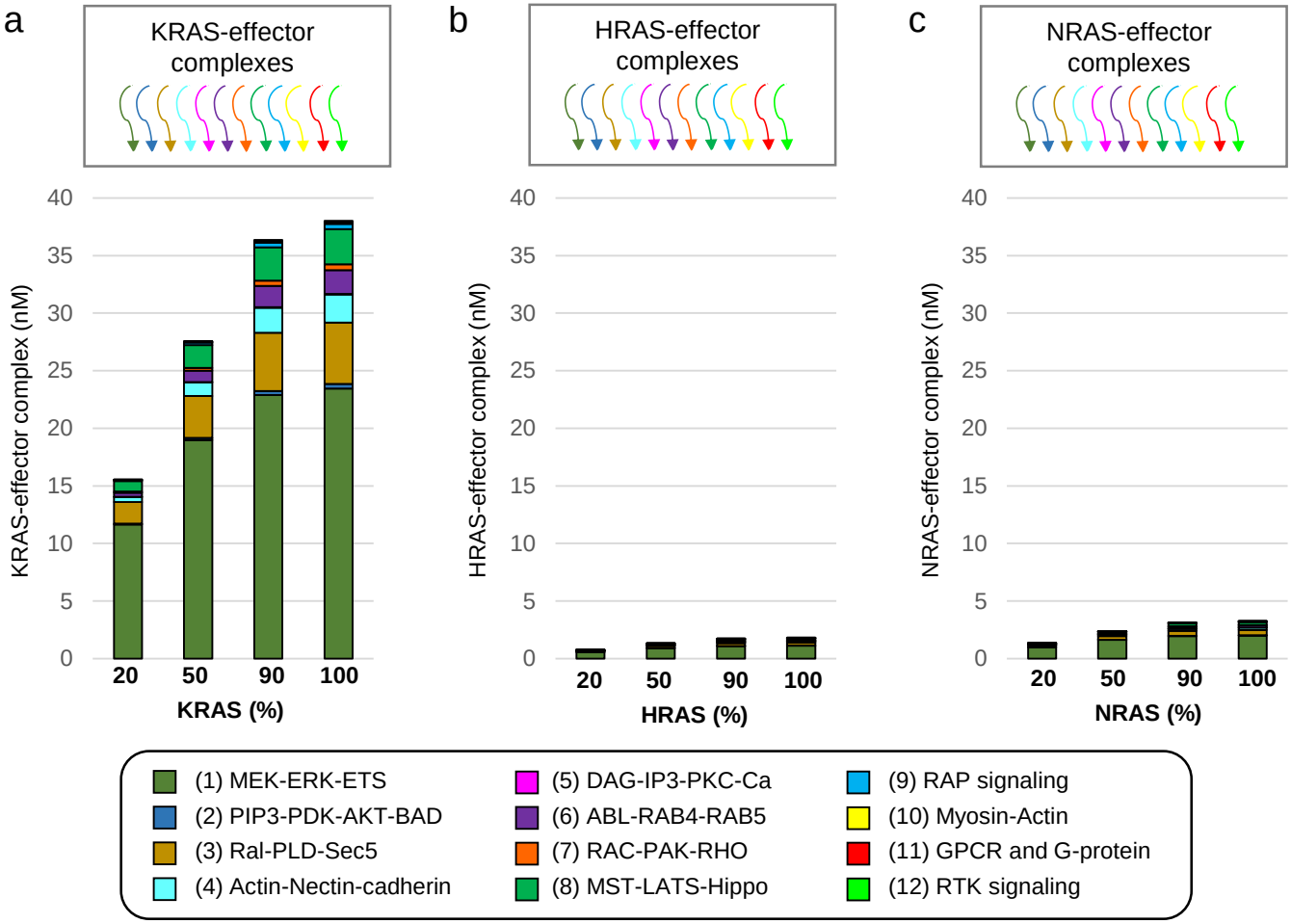

Figure S3. Results of the equilibrium network analysis of nano molar concentrations of Ras-effector complexes in colon tissue separately for KRAS- (**a**), HRAS- (**b**), and NRAS- (**c**) effector complexes. The complex formations were calculated using increasing amounts of KRAS (and HRAS/NRAS, respectively) with 100% corresponding to the total concentration for mimicking a 100 % load of GTP. The percentage of KRAS is complex with effectors was calculated as the fraction compared to the total RAS complexes (sum of HRAS, KRAS, and NRAS effector complexes): ~88%.

a

RAS subfamily	Effectors	Pathways
HRAS KRAS NRAS	▪ UB-like domains	Proliferation/self-renewal, differentiation, adhesion, migration, apoptosis, cytoskeleton
RRAS RRAS2 MRAS		Cytoskeleton, (weak) MAP kinase activator
RAP1A RAP1B RAP2A RAP2B RAP2C	▪ FERM domains ▪ Talin, N-terminal F0 domain	Integrin-mediated cell adhesion, cell spreading
RALA RALB	▪ IPT domains ▪ Coiled-coil	Protein trafficking, differentiation, cytoskeleton
RIT1 RIT2		Neuronal survival
ERAS		Restricted to embryonic stem cells
DIRAS1 DIRAS2 DIRAS3		?
RASD1 RASD2		Nitric oxide signaling
NKIRAS1 NKIRAS2		NF-kappa-beta signaling
REM1 REM2 RRAD		Voltage-gated $Ca2^{+}$ channels
GEM RERG RERGL RASL10A RASL10B RASL11A RASL11B RASL12		?
RHEB RHEBL1		mTOR signaling

RAS (onco) proteins

b

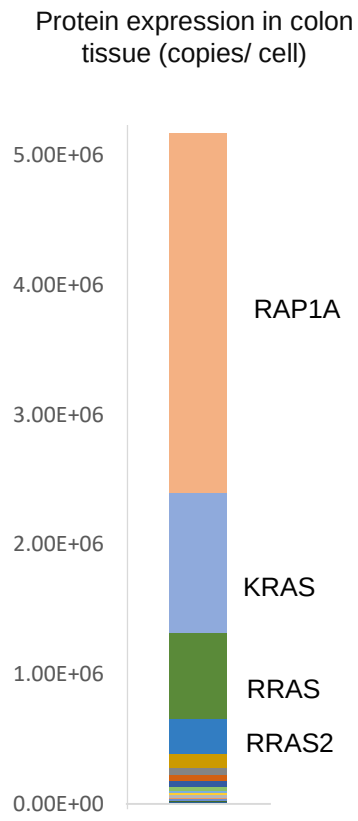

Figure S4. Members of the Ras subfamily, effector domains, functional annotations, and expression in colon tissue. (**a**) Functional annotations and pathways are summarized based on Colicelli 2004, analyses performed in this manuscript, and Uniprot (https://www.uniprot.org/). (**b**). Protein expression (copies/cell) in colon tissue from Wang et al 2019.

Reference:
Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004 Sep 7;2004(250):RE13.

Wang D, Eraslan B, Wieland T, Hallström B, Hopf T, Zolg DP, Zecha J, Asplund A, Li LH, Meng C, Frejno M, Schmidt T, Schnatbaum K, Wilhelm M, Ponten F, Uhlen M, Gagneur J, Hahne H, Kuster B. A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. Mol Syst Biol. 2019 15:e8503.

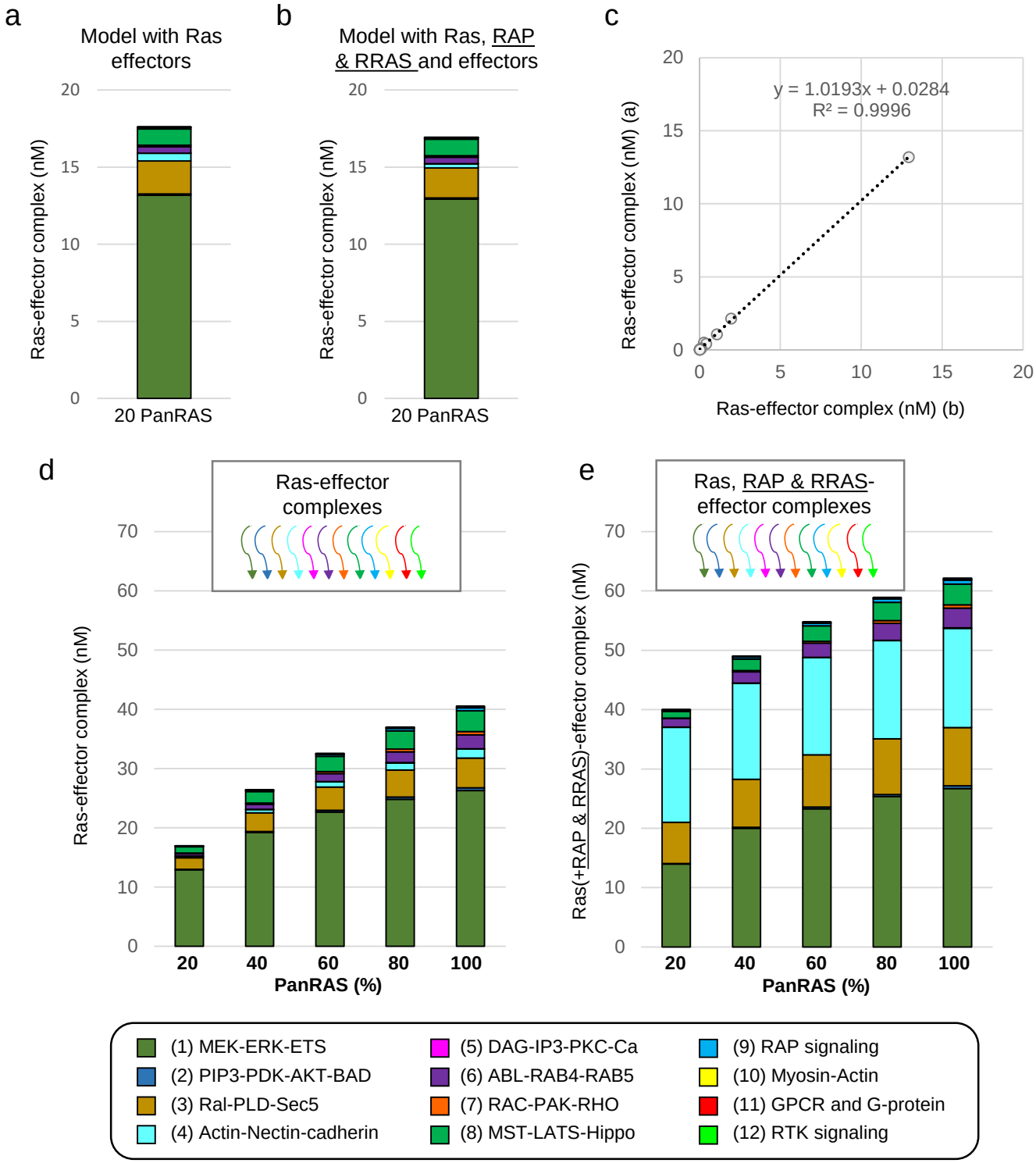

Figure S5. Results of the equilibrium network analysis of nano molar concentrations of Ras-effector complexes in colon tissue comparing two types of network models. (**a**) Ras-effector complex formations calculated at 20% total Ras for the network model including only Ras proteins and 56 effectors. (**b**) Ras-effector complex formations calculated at 20% total Ras for the network model including Ras proteins and 56 effectors, and additionally experimentally confirmed interactions of a subset of Ras effectors with RAP and RRAS. (**c**) Correlation of the 12 Ras effector group complex concentrations in normal colon tissue (with 20 % active Ras) comparing the results of the two networks models shown in panels (a) and (b). (**d**) Ras-effector complex formations calculated at increasing total Ras for the network model including only Ras proteins and 56 effectors. (**e**) Ras-effector complex formations calculated at increasing total Ras for the network model including Ras proteins and 56 effectors, and additionally experimentally confirmed interactions of a subset of Ras effectors with RAP and RRAS.

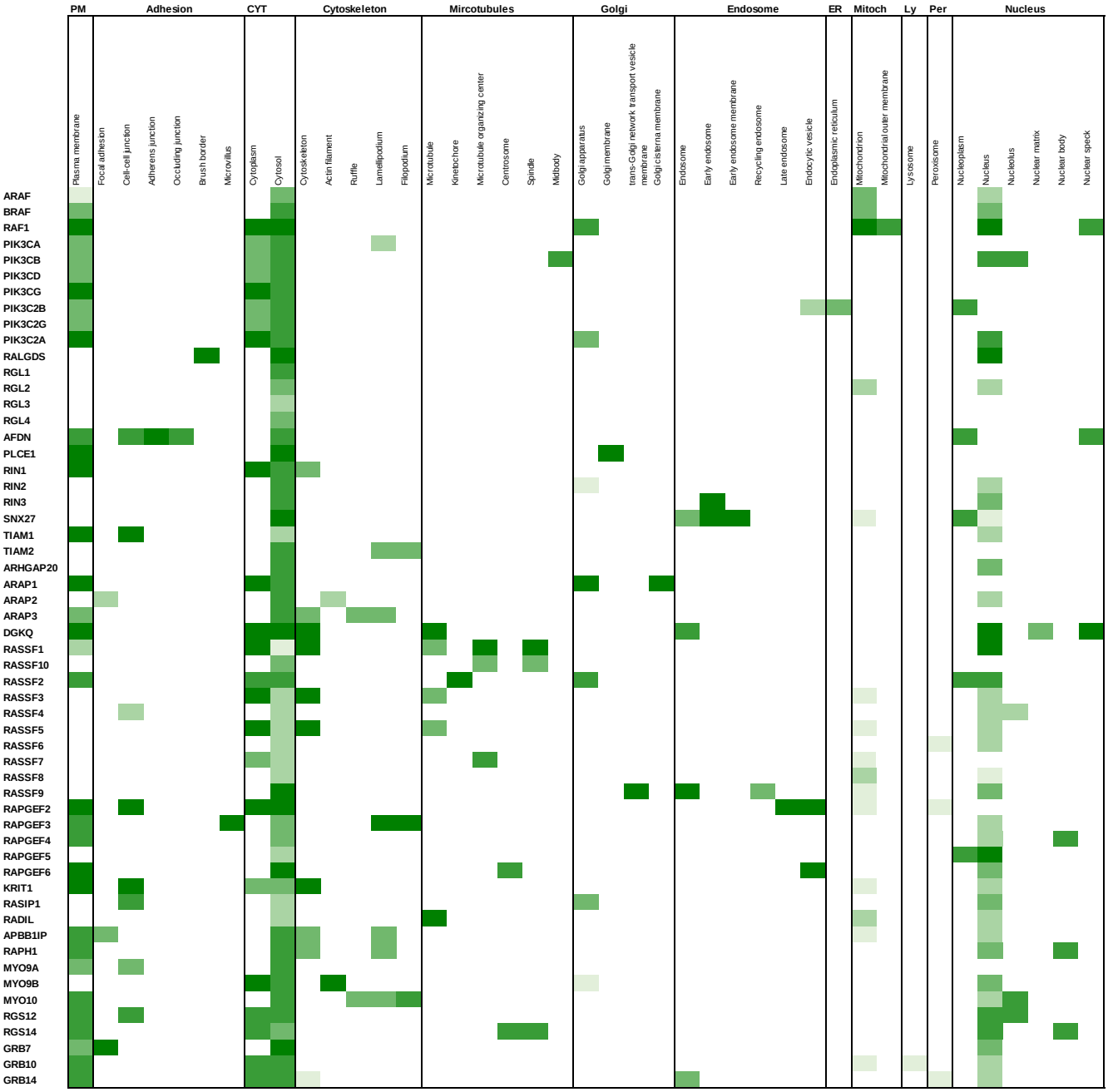

Figure S6. Information about the subcellular information of the 56 Ras-effectors obtained from the COMPARTMENTS database (https://compartments.jensenlab.org/Search). The confidence score associated to a particular localisation ranges from low confidence (0) (white) to high confidence (5) (dark green).

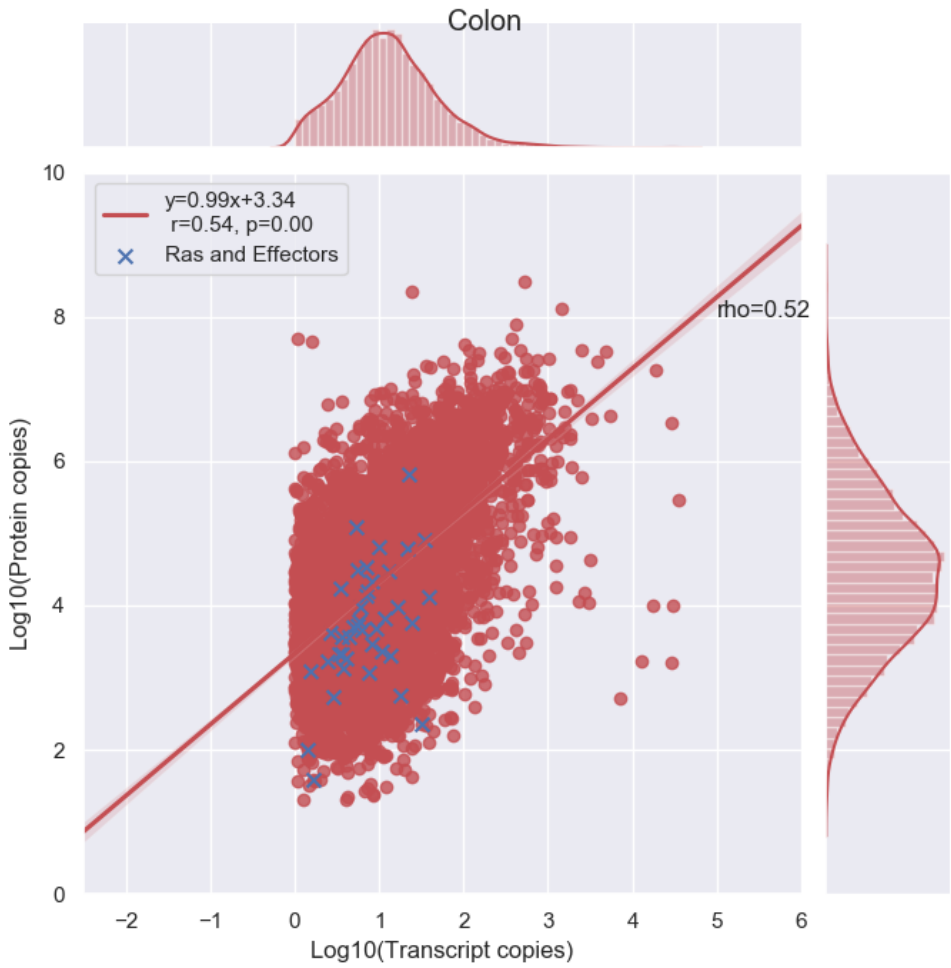

Figure S7. Spearman correlation between transcript and protein expression in colon tissue based on Wang et al, 2019. Ras and effectors are indicated with a blue cross. The mean linear regression error is 0.697.