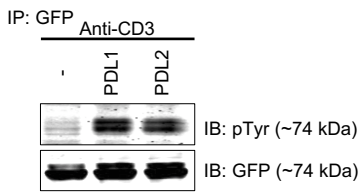
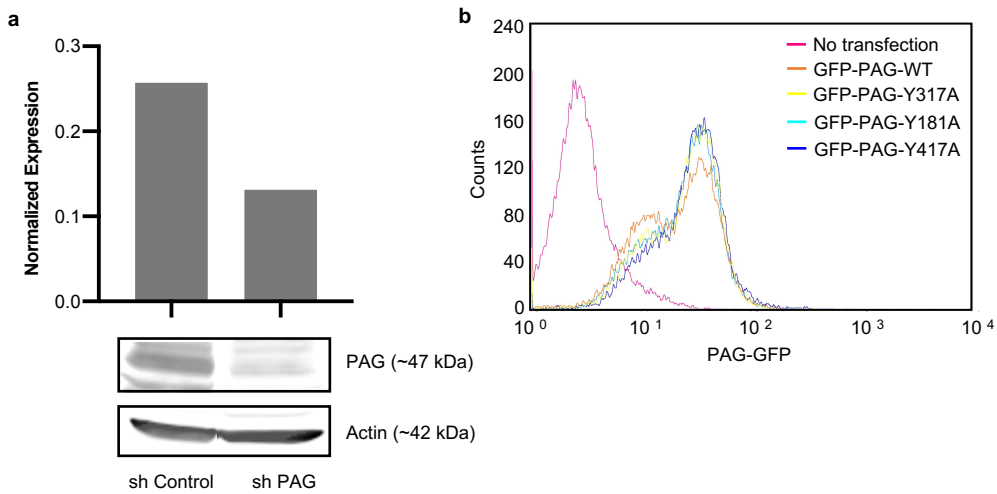


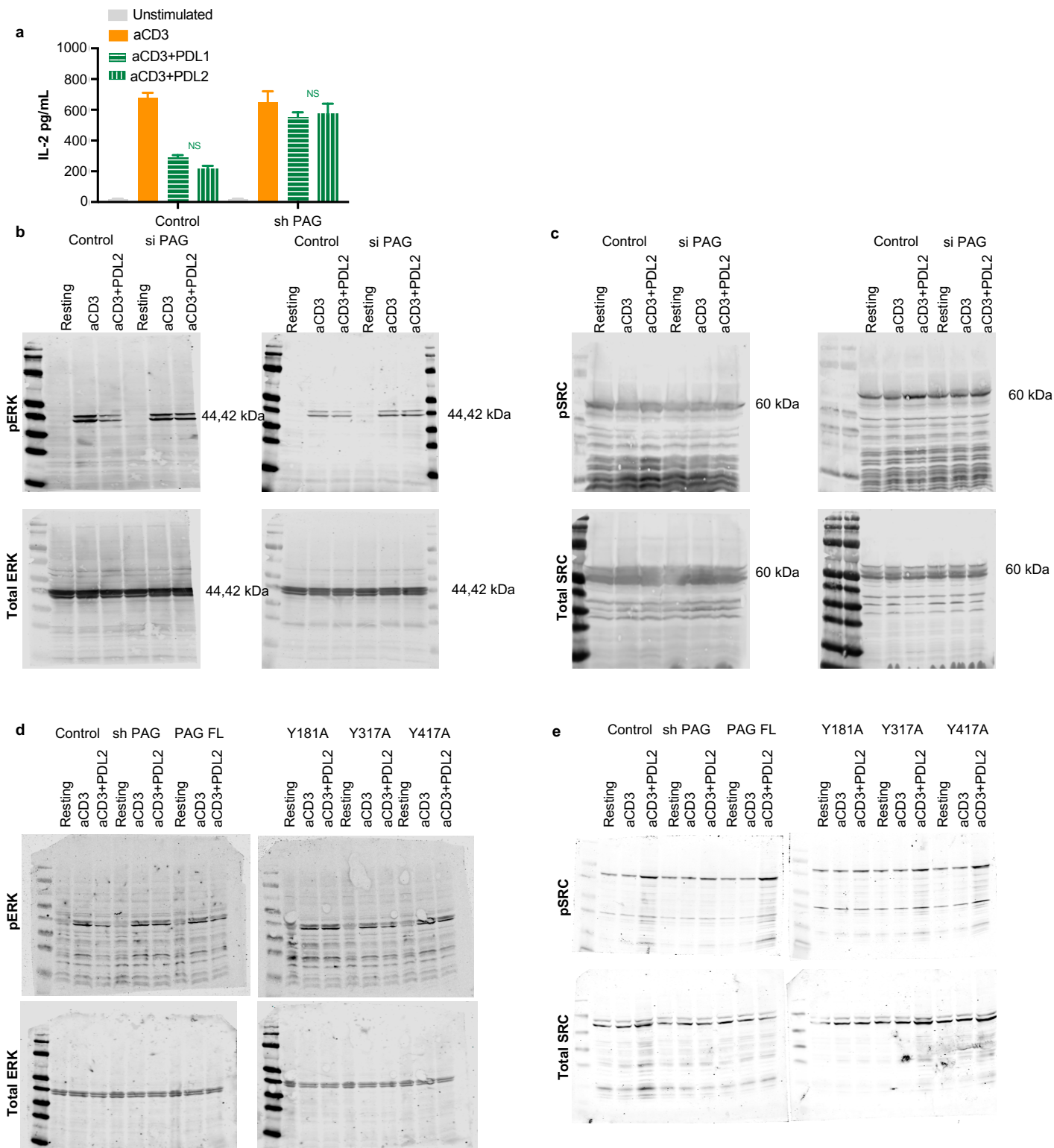
Supplementary Figure 1. PAG protein expression in the top 10 highest expressing tissues¹⁴.



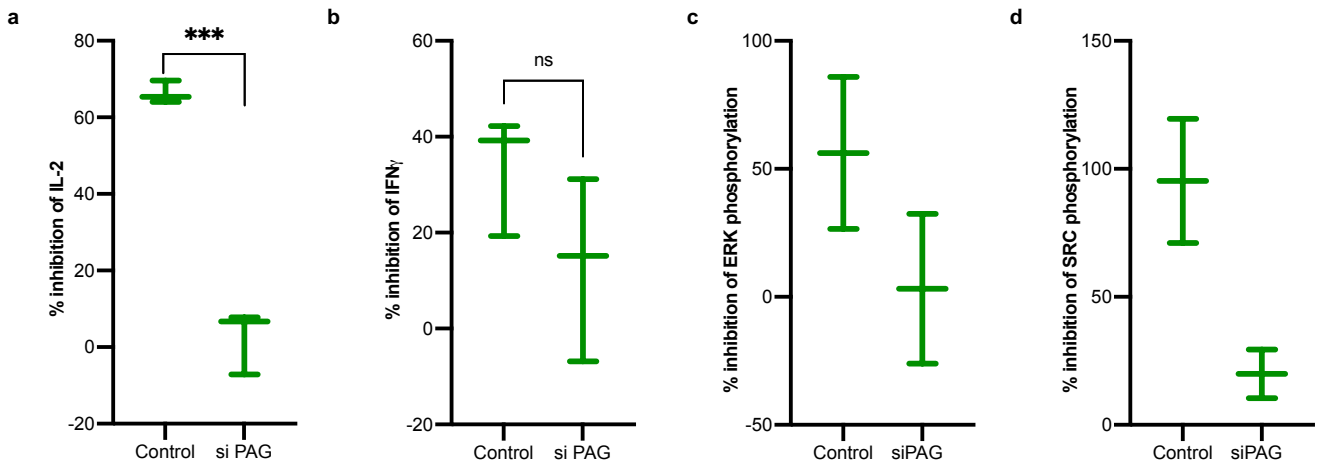
Supplementary Figure 2. Jurkat cells expressing PAG-GFP were stimulated with anti-CD3 alone or with PDL1 or PDL2. PAG-GFP was enriched by immunoprecipitation of the lysates and phosphotyrosine was detected by immunoblot. Representative blot of three independent experiments is shown.



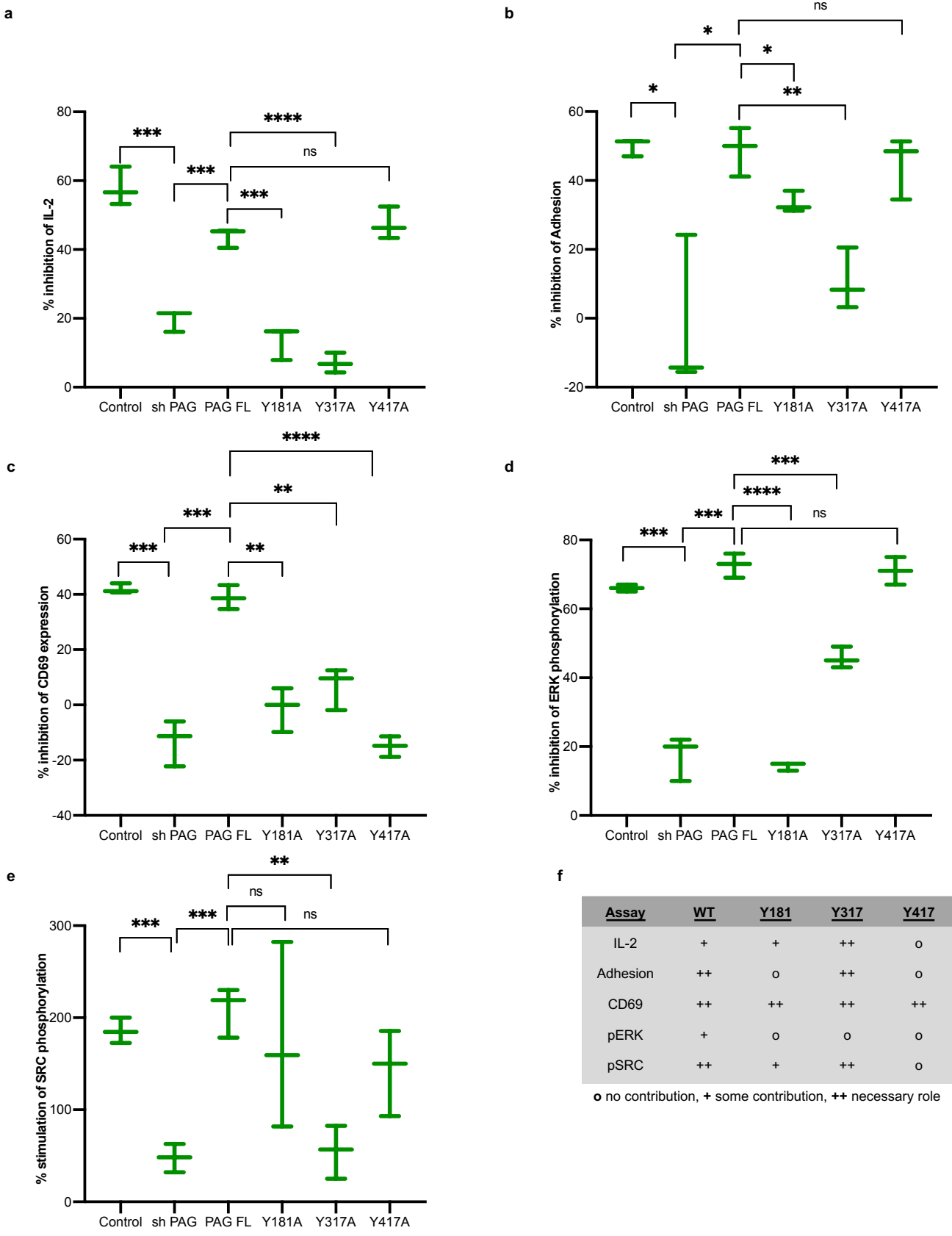
Supplementary Figure 3. a. Jurkat cells expressing either a non-targeting shRNA (shControl) or a PAG targeting shRNA (shPAG) were lysed and run on a western blot. Immunoblot shows PAG and actin protein levels. Representative blot is shown. **b.** Jurkat cells expressing sh PAG were transfected with wild type full length PAG or phosphodeficient versions and GFP expression was quantified by flow cytometry.



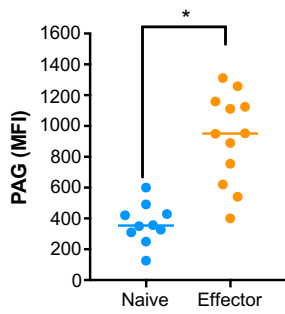
Supplementary Figure 4. **a.** ELISA of secreted IL-2. Jurkat cells expressing sh Control or sh PAG were stimulated for 24 hours before supernatants were collected. Bars represent mean \pm SEM of three independent experiments. **b - e.** Representative western blots of primary T cells (**b, c**) and Jurkat cells (**d, e**) expressing si/sh control or si/sh PAG and rescue transfections with various versions of PAG into shPAG expressing Jurkat cells as indicated. **d.** Table summarizing the contributions of phosphotyrosines 181, 317, and 417 to the assessed T cell functions and PD-1 signaling.



Supplementary Figure 5. a. and b. ELISA of secreted IL-2 (a) and IFN γ (b) in the supernatants of primary human CD3⁺ T cells collected 48 hours following stimulation by magnetic beads. T cells either expressed a non-targeting siRNA (control) or PAG targeting siRNA pool (siPAG). **c and d.** Phosphorylated ERK (c) and phosphorylated SRC (d) were detected by western blot of human CD3⁺ T cell lysates 5 minutes after stimulation by magnetic beads. Fold change is calculated relative to anti-CD3 stimulation (c) or unstimulated (d). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns not significant.



Supplementary Figure 6. a. ELISA of secreted IL-2 in the supernatants of Jurkat cells collected 24 hours following stimulation by magnetic beads. Jurkat T cells either expressed a non-targeting shRNA (control) or PAG targeting shRNA (shPAG). For rescue transfections, shPAG Jurkats were transiently transfected with full length, wild type PAG (PAG FL) or with phosphodeficient mutants (Y181A, Y317A, Y417A). b. Adhesion assay of Jurkat cells to fibronectin following stimulation for 15 minutes. The number of adherent cells remaining, expressed as a percentage of the total number of labeled cells, was determined with a fluorescent plate reader. PAG knockdown and rescue transfections as in (A). c. Percentage of Jurkat cells expressing CD69 on the surface following 24 hour stimulation, measured by flow cytometry. PAG knockdown and rescue transfections as in (A). d and e. Phosphorylated ERK (D) and phosphorylated SRC (E) were detected by western blot of Jurkat lysates 5 minutes after stimulation by magnetic beads. PAG knockdown and rescue transfections as in (A). % inhibition is calculated by $100 - \text{CD3+PDL2} \div \text{CD3} \times 100$. Bars represent the mean \pm SEM of three independent experiments. f. Table summarizing the contribution of each phosphotyrosine position to individual T cell functions assayed. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns not significant.



Supplementary Figure 7. Human naïve ($CD8^+CCR7^+CD62L^+$) and effector ($CD8^+CCR7^-CD62L^-$) T cells were stained with intracellular anti-PAG antibody and analyzed by flow cytometry. MFI - median fluorescent intensity.

Supplementary Table 1. IL-2 ELISA

Primary human T cells	Unstimulated			aCD3			aCD3+PDL2		
Control	20	25	0	661	640	655	201	230	227
si PAG	20	30	32	553	578	610	510	619	569

Jurkat cells	Unstimulated			aCD3			aCD3+PDL2		
Control	20	5	23	805	946	902	289	410	422
sh PAG	35	22	32	871	953	944	731	748	741
PAG FL	25	23	23	754	835	810	411	457	482
PAG Y181A	19	27	15	750	848	821	691	710	688
PAG Y317A	12	14	27	737.8349	790	780	688	756	702
PAG Y417A	33	18	29	777	810	838	440	435	398

Supplementary Table 2. IFNg ELISA

	Unstimulated			aCD3			aCD3+PDL2		
Control	27	71	46	1298	1301	1514	750	1050	920
si PAG	154	30	36	1650	1453	1404	1400	1000	1500

Supplementary Table 3. Percent inhibition of CD69 expression

	Unstimulated			aCD3			aCD3+PDL2		
Control	3	3	0	51	59	54	30	33	32
sh PAG	3	4	5	54	62	67	66	69	71
PAG FL	7	6	6	49	57	53	32	35	30
PAG Y181A	8	6	2	51	45	50	56	45	47
PAG Y317A	7	6	11	52	56	54	47	49	55
PAG Y417A	6	7	10	27	35	32	31	39	38

Supplementary Table 4. Percent inhibition of adhesion

	Unstimulated			aCD3			aCD3+PDL2		
Control	10	14	11	35	37	34	17	18	18
sh PAG	14	18	12	28	32	33	32	37	25
PAG FL	8	10	11	30	38	34	15	17	20
PAG Y181A	9	13	7	32	27	31	22	17	21
PAG Y317A	12	16	14	31	36	34	30	33	27
PAG Y417A	8	13	17	37	29	33	18	19	17

Supplementary Table 6. B16F10 Tumor Measurements

Days	WT										WT + Anti-PD-1									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	0										0									
2	0										0									
3	0										0									
4	0										0									
5	0										0									
6	0										0									
7	0										0									
8	62.5	72	108	75	43						42	63	51							
9	85	100	100	126	97						107	126	97							
10	110	133	110	140	167						150	105	128							
11	133	128	143	270	236						203	155	175							
12	158.4375	155	169	408	386.334						239.0625	288	340							
13	251	178	207	455	420	61.3	48.4	48.8			328	352	397	67.6	58	0				
14	354	301	360	573	298						268	494	445							
15	465.75	710	698	606	564.9188						524	627	521							
16	768	893	1057	725	615						624	757	674							
17	1102	1205	1108	947	799	171.5	162	486			795	827	973	288	196	0				
18	1547	1403	1650	1350	1220						1187	1080	1280							
19	1688	1620	2017	1505	1521	744.2	635	726			1071	1231	1533	299.2	282.8	0				
20	1801	2017	2219	2403	2101						1371	1775	1895							
21	3105	2258	2730	2437	2450	1890	1793.4	2073.6	1852	2280	2364	1428.8	2229.2	0						

Days	PAG KO										PAG KO + Anti-PD-1									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	0										0									
2	0										0									
3	0										0									
4	0										0									
5	0										0									
6	0										0									
7	0										0									
8	154.791	125	117	108	75						41	37	66							
9	180	140	170	127	64						55	58	73							
10	163	145	199	150	76						73	112	101							
11	170	145	213	178	99						90	179	144							
12	179.5625	91	218	253.6285	110.808						141	250	204							
13	202	155	287	288	186	15.4	76.3	35.3			172	246.63	119.88	30.4	27.2	19.2				
14	240	206	310	310	265						223	212.84	193.98							
15	285	179.5625	378.895	324	313.8266						270	249.77	250							
16	351	363	420	441	501						274	266	289							
17	527	450	558	600	681	64	484.3	766.1			180	301	321	395.1	423.2	115				
18	596	636	753	633	789						455	447	305							
19	750	842	831	1048	1190	136.1	465.8	1023.4			603	460	337	885.8	617.2	180				
20	1090	1210	1258	1330	1644						762	952	631							
21	1860	1407	1548	1472	1950	727.4	738.1	1502.2	950	1308	903	1381.8	1264.8	1140.8						

