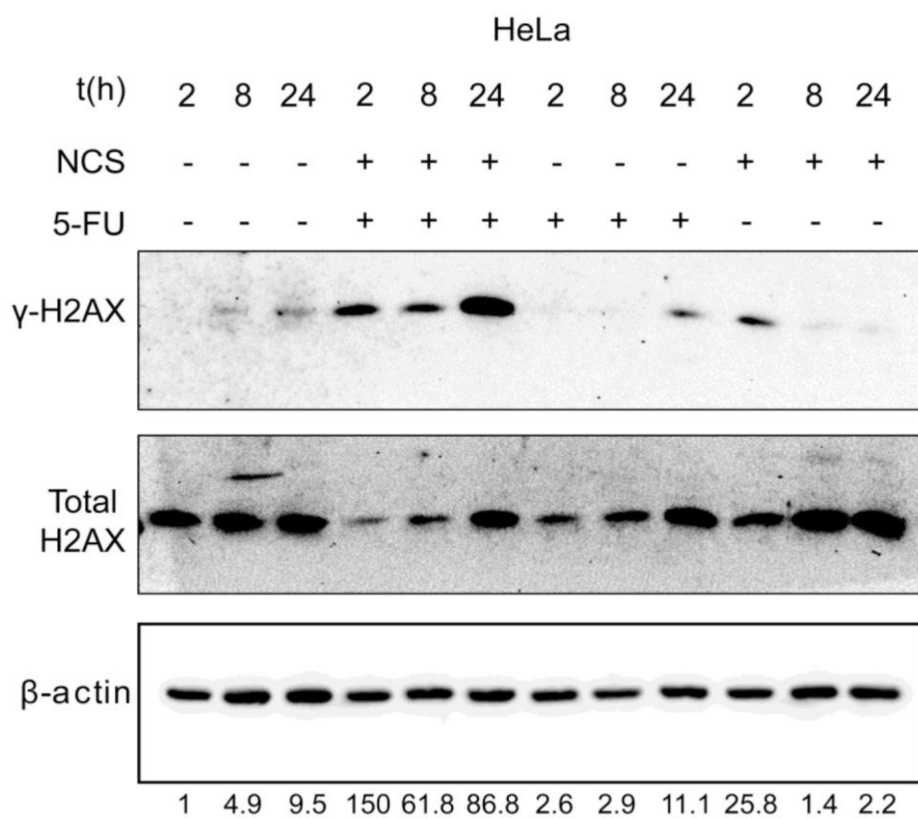
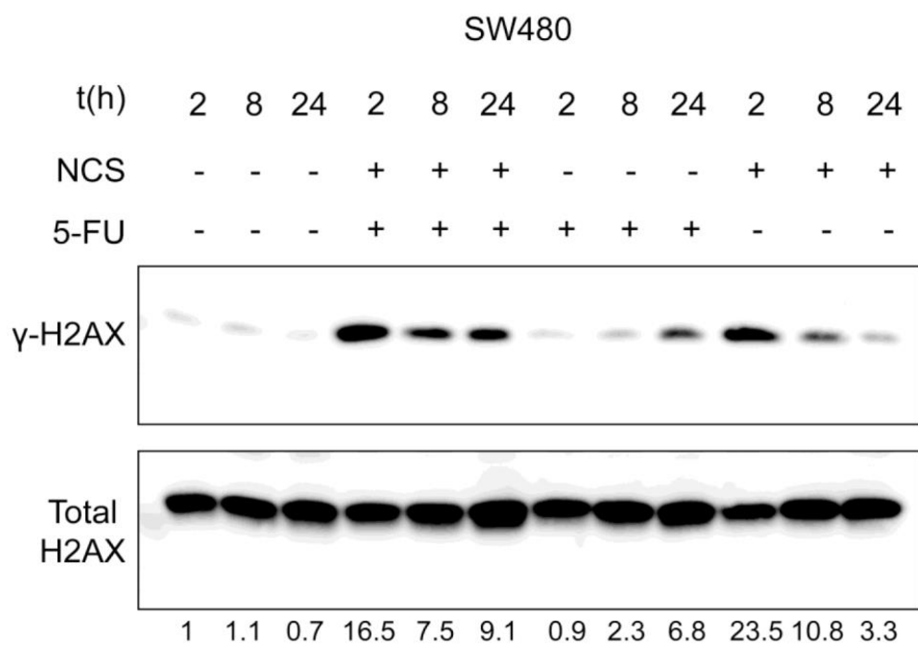
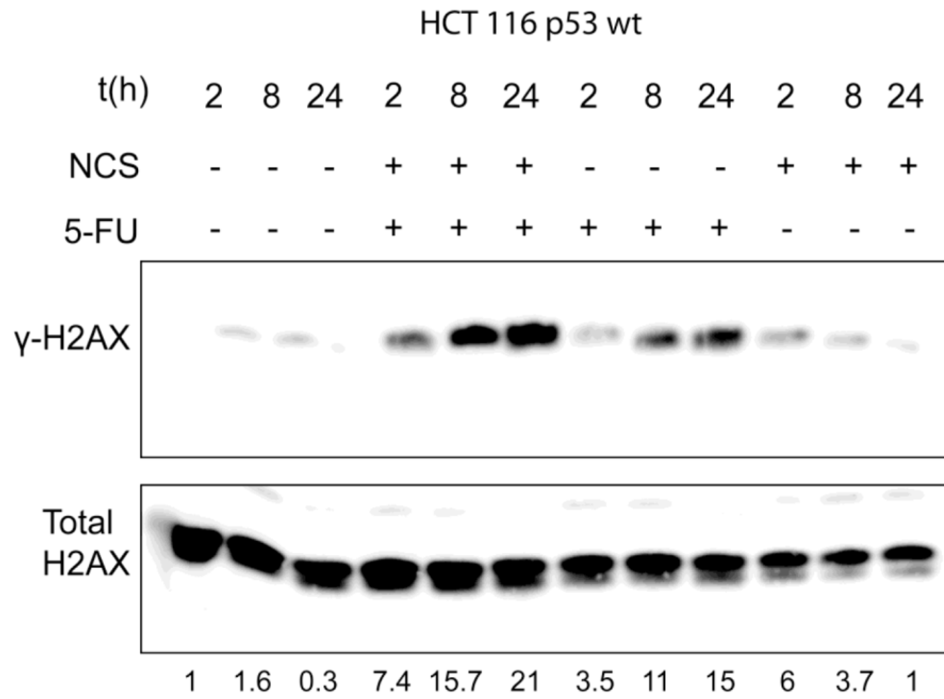
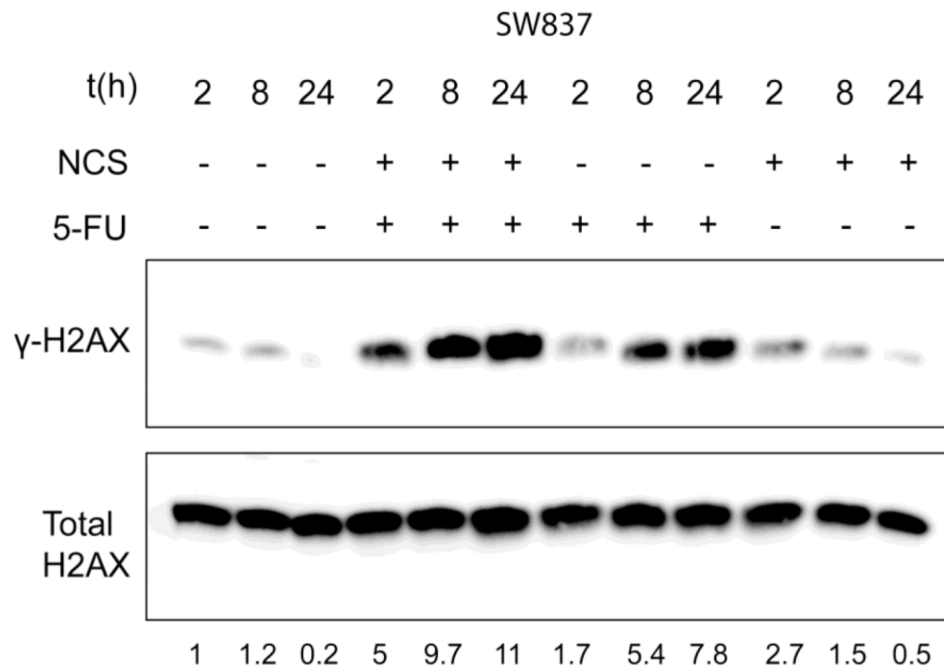
5-Fluorouracil sensitizes colorectal tumor cells towards double stranded DNA breaks by interfering with homologous recombination repair

Supplementary Material

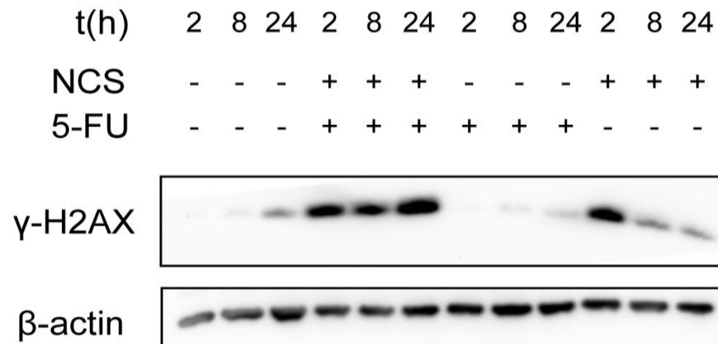

(A)
HeLa
t(h) 2 8 24 2 8 24 2 8 24 2 8 24
NCS - - - + + + - - - + + +
5-FU - - - + + + + + + - - -
γ-H2AX
Total H2AX
β-actin
1 4.9 9.5 150 61.8 86.8 2.6 2.9 11.1 25.8 1.4 2.2


(B)
SW480
t(h) 2 8 24 2 8 24 2 8 24 2 8 24
NCS - - - + + + - - - + + +
5-FU - - - + + + + + + - - -
γ-H2AX
Total H2AX
1 1.1 0.7 16.5 7.5 9.1 0.9 2.3 6.8 23.5 10.8 3.3

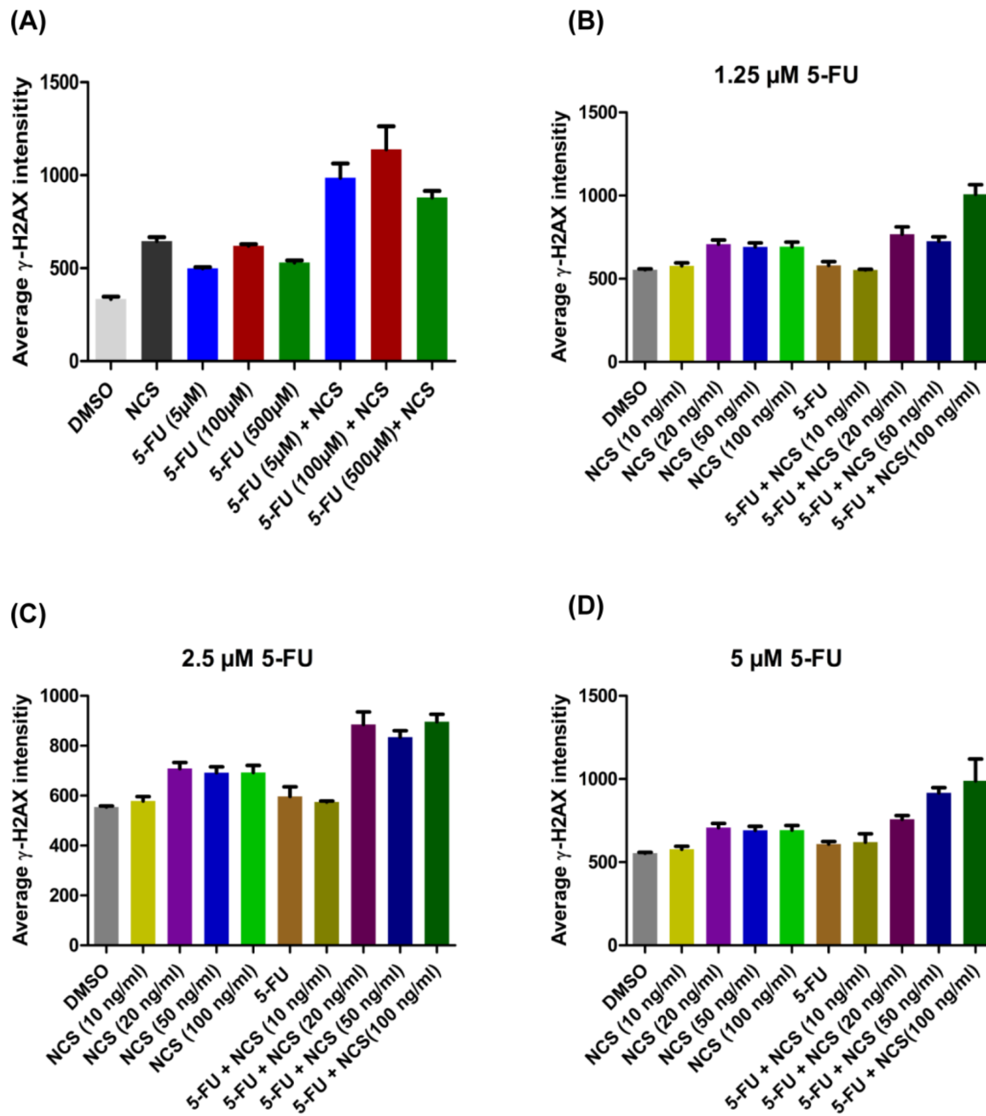

(C)
HCT 116 p53 wt
t(h) 2 8 24 2 8 24 2 8 24 2 8 24
NCS - - - + + + - - - + + +
5-FU - - - + + + + + + - - -
γ-H2AX
Total H2AX
1 1.6 0.3 7.4 15.7 21 3.5 11 15 6 3.7 1


(D)
SW837
t(h) 2 8 24 2 8 24 2 8 24 2 8 24
NCS - - - + + + - - - + + +
5-FU - - - + + + + + + - - -
γ-H2AX
Total H2AX
1 1.2 0.2 5 9.7 11 1.7 5.4 7.8 2.7 1.5 0.5

(E) SW620

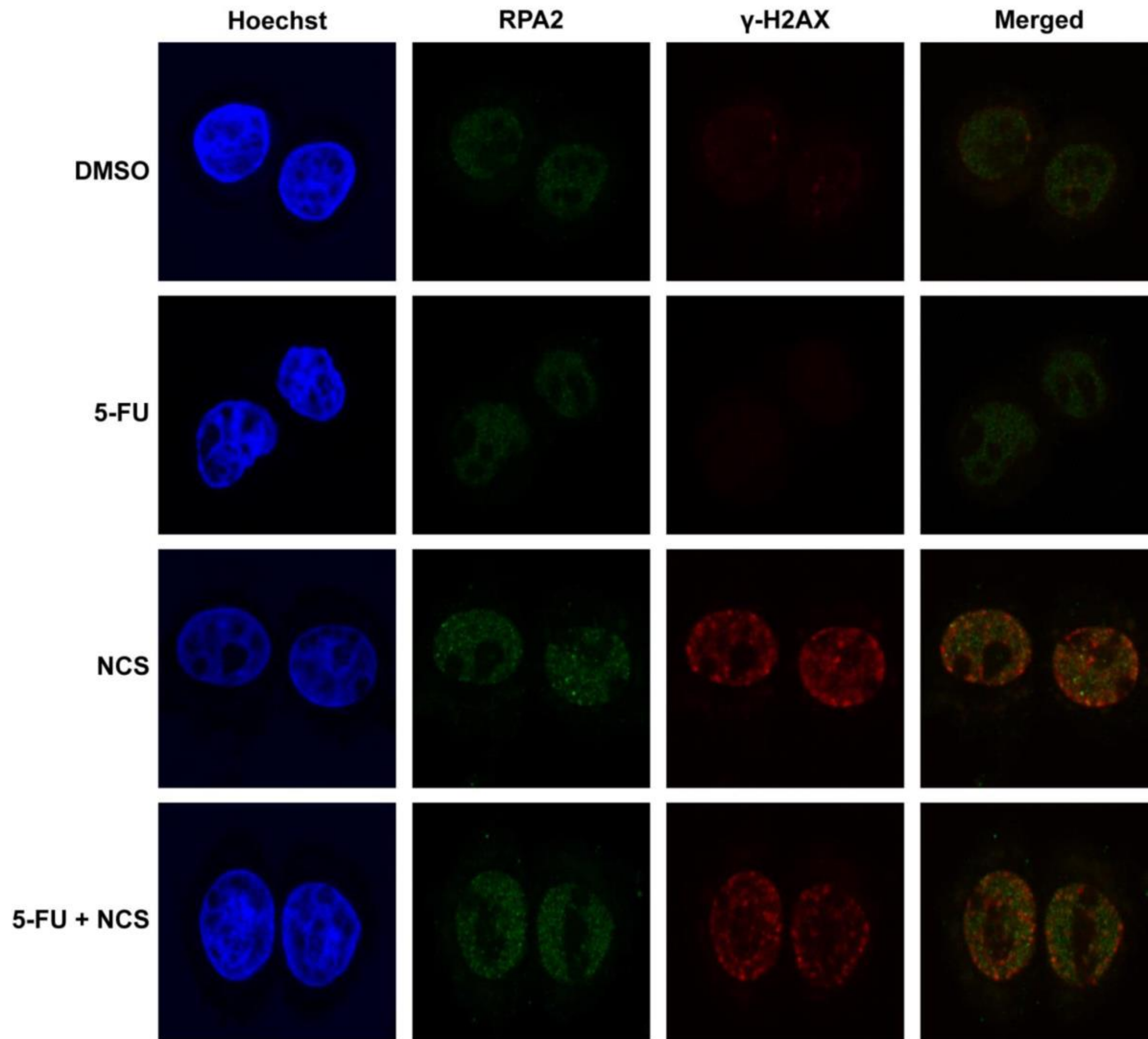


Supplemental Figure S1. **5-FU in combination with NCS leads to persistent γ-H2AX accumulation.** Cells were first incubated with 5 μM 5-FU for 24 h followed by treatment with 100ng/ml NCS, and then harvested at the indicated time points. (A) HeLa, (B) SW480, (C) HCT116 p53 wt, (D) SW837 and (E) SW620, cells were treated as described with 5-FU and NCS. Whole cell extracts were immunoblotted, followed by detection of phosphorylated H2AX (γ-H2AX), total H2AX (for normalization of γ-H2AX) and β-actin (loading control).

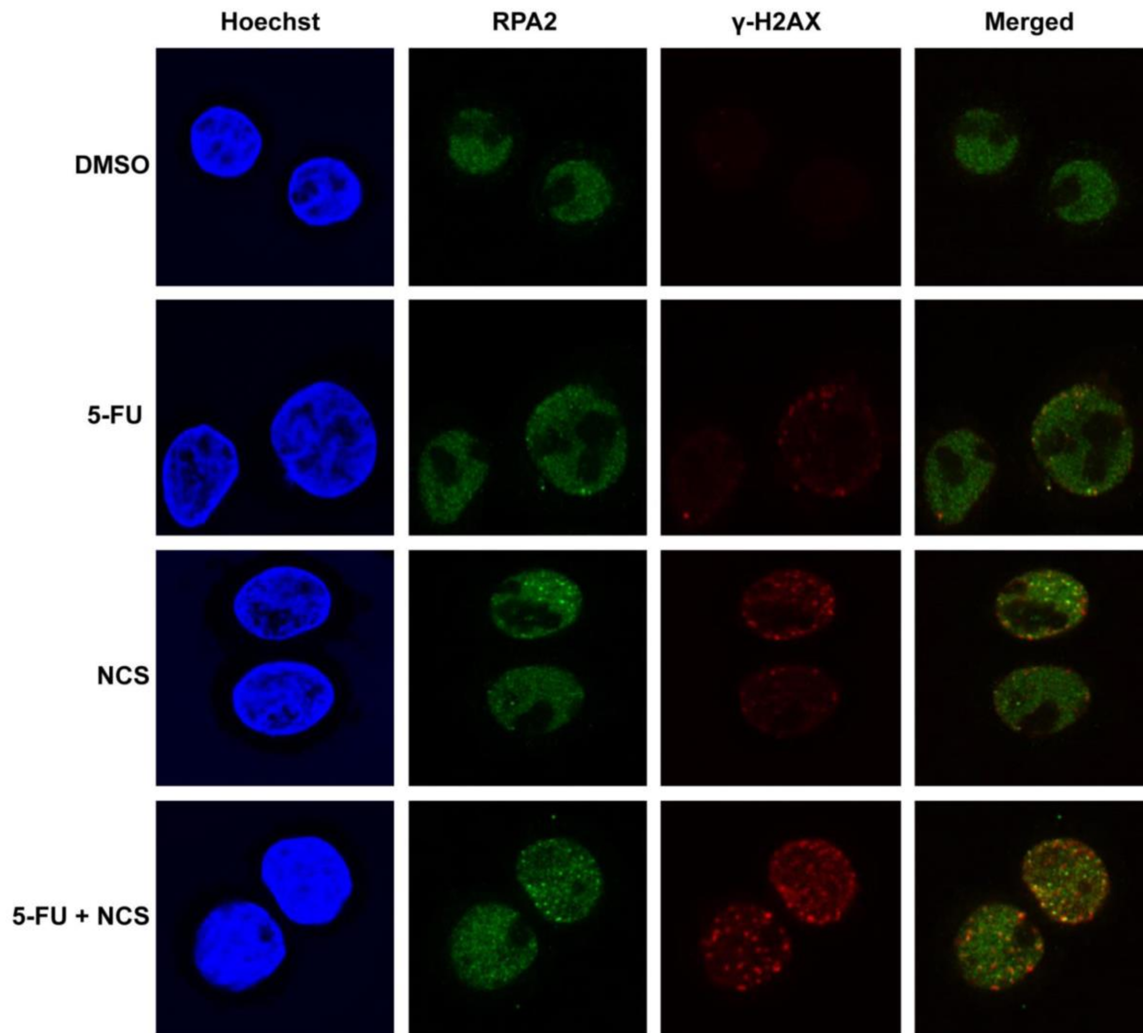


Supplemental Figure S2. Dose-dependence of DNA damage upon treatment with 5-FU and NCS. SW480 cells were treated with (A) 100 ng/ml NCS and 5, 100 or 500 μM 5-FU, (B) 1.25 μM 5- FU and 100, 50, 20 or 10 ng/ml NCS, (C) 2.5 μM 5-FU and 100, 50, 20 or 10 ng/ml NCS, (D) 5 μM 5-FU 100, 50, 20 or 10 ng/ml NCS. The cells were treated with the indicated 5-FU concentrations for 24 h, followed by NCS plus 5-FU for 24 h. Cells treated with DMSO were used as negative controls. The γ-H2AX intensities were determined in single cells by immunofluorescence and automated microscopy. The fluorescence intensities of at least 1000 cells per sample were determined by digital image analysis and plotted as the mean and standard error mean of three experiments.

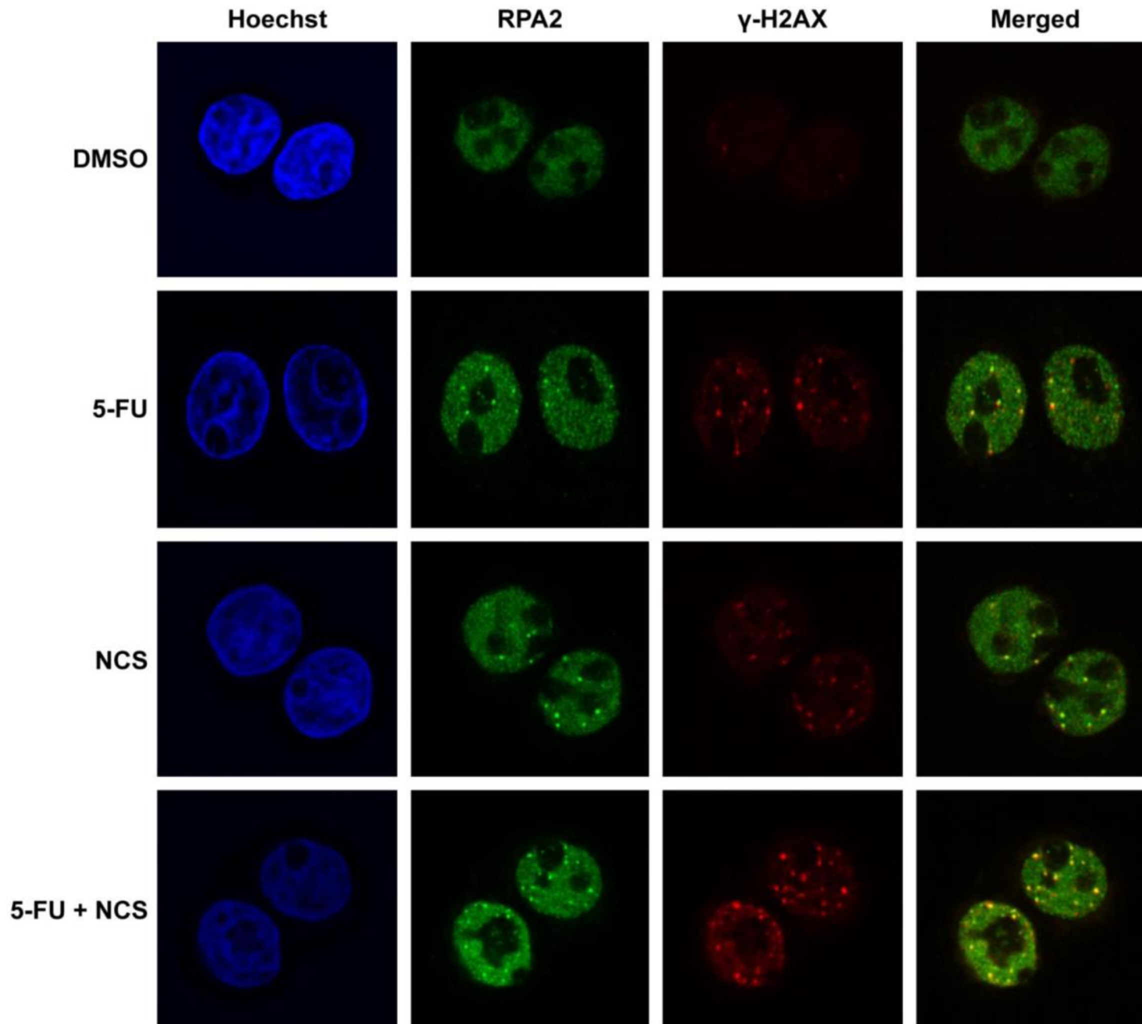
(A)



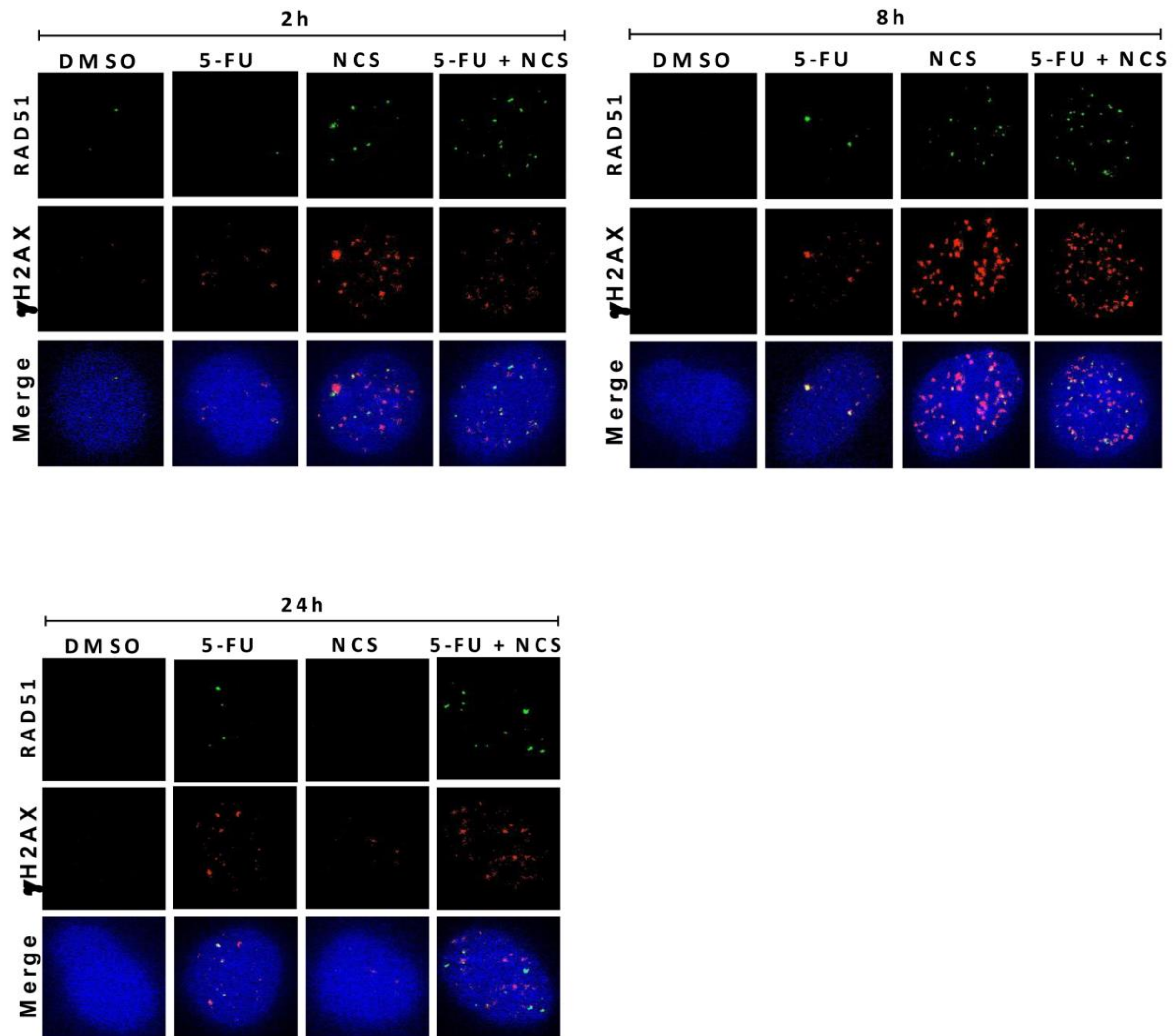
(B)



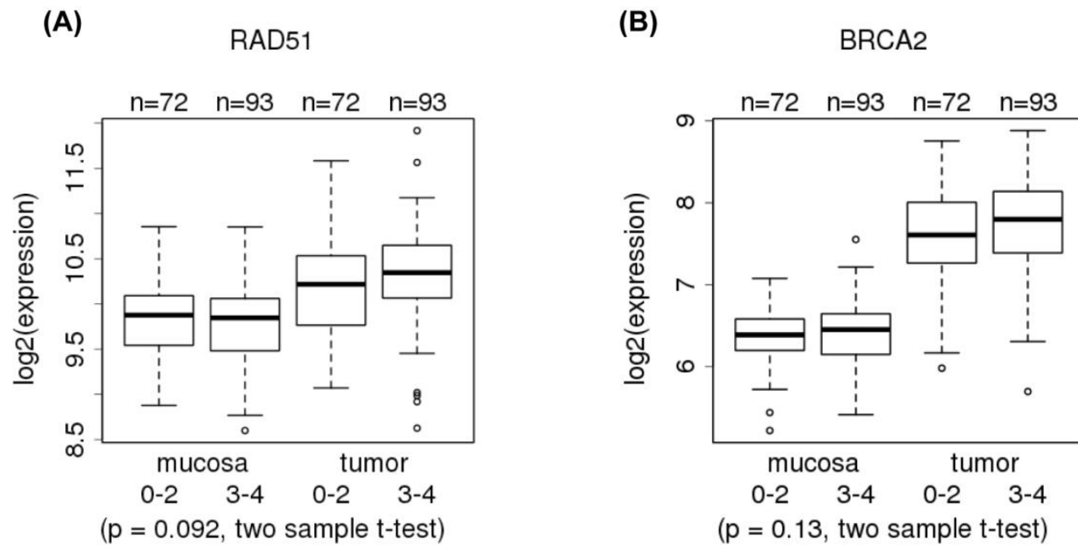
(C)



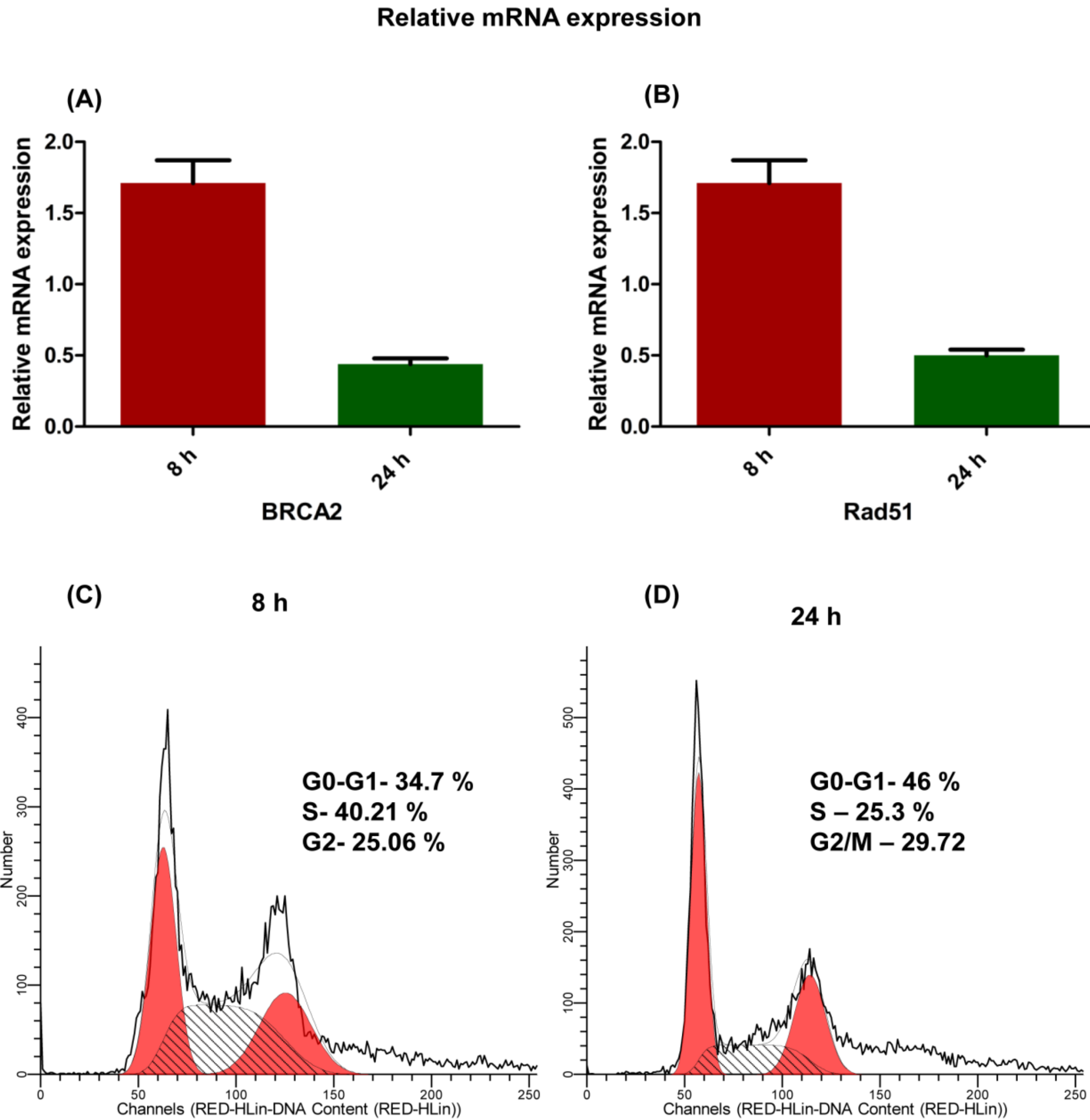
Supplemental Figure S3. 5-FU and/or NCS do not decrease the recruitment of RPA2. Confocal microscopy images of SW480 cells treated with DMSO, 5 μM 5-FU + 100ng /ml NCS, 5 μM 5-FU, or 100 ng/ml NCS for (A) 2 h, (B) 8 h and (C) 24 h post NCS treatment, followed by IF staining of RPA2 and phospho-H2AX.



Supplemental Figure S4. Accumulation of Rad51 and phospho-H2AX foci upon treatment with 5-FU and/or NCS. SW480 cells were treated and subjected to immunofluorescence analysis as described in Fig. 3, panel C. Separate image files are shown to document the localization of Rad51 and γ-H2AX, and merged images show the partial colocalization of the two.



Supplemental Figure S5. **Expression of Rad51 and BRCA2 in human biopsies.** mRNA expression of HRR components in colorectal cancers and normal mucosa, evaluated in biopsy material from patients using microarray hybridization, and classified according to tumor grade; (A) Rad51 and (B) BRCA2.



Supplemental Figure S6. Increased mRNA expression of RAD51 and BRCA2 in S phase SW480 cells were synchronized using a double thymidine block and then released by removing excessive thymidine. The cells were harvested 8h and 24 h post release, and (A) Rad51 as well as (B)BRCA 2 mRNA levels were analyzed by quantitative RT-PCR and normalized to the reference gene GAPDH. Parallel propidium iodide staining of the cells and analysis by flow cytometry document an enrichment for S phase at 8 h (C) post release and a normal distribution of the DNA content at 24 h post release (D).

patient	cancer detailed location	gender	age_at_pathologic_diagnosis_by_biopsy_months	age_at_surgery_months	time_to_progression_months	dfs_event	tumor_grade_at_surgery	RAD51 mucosa	RAD51 tumor	BRCA2 mucosa	BRCA2 tumor
Pat-JG2		FEMALE	663	667	0.0000000	TRUE	3	9.93271524271006	9.4802183153759	6.3207502627855	6.88921816365709
Pat-JG4		MALE	783	787	40.2472330	FALSE	4	9.98729358974371	11.0624710704209	6.76901977975552	7.30007861171228
Pat-JG6		MALE	747	752	29.6022506	FALSE	4	9.30417240819116	10.0218374139692	6.3133576353198	7.8679212132719
Pat-JG8		MALE	737	742	32.6248999	FALSE	4	9.3157264466179	10.4315478279654	6.04772817476927	8.17713894890417
Pat-JG10		FEMALE	621	625	31.5735436	FALSE	4	9.36872244067524	10.2957877158105	5.66377074932453	7.69047666179671
Pat-JG12		MALE	706	709	34.4976283	FALSE	2	9.39162488785565	10.5833731474297	6.3277349589064	8.2808777204461
Pat-JG14		MALE	843	847	32.6577548	FALSE	4	10.1179988190345	11.1748265004169	5.78048749685958	7.71506988546085
Pat-JG16		MALE	723	727	30.2922031	FALSE	3	9.68299308989286	10.7428849346383	6.62471156380279	8.22605305954173
Pat-JG18		MALE	656	660	28.0580710	FALSE	2	9.71652006915316	10.5211490270596	6.24556446551268	7.00267715299219
Pat-JG20		MALE	633	637	27.5323929	FALSE	3b	9.93773125163652	9.96731054193543	6.08705501533867	7.44525085048825
Pat-JG22		MALE	601	NA	NA	NA	4	9.35131075560352	10.665993515512	6.52667352390912	8.3969968889138
Pat-JG24		MALE	698	702	14.9161174	FALSE	3	9.59710645042617	10.9196329992415	6.03209814773894	6.79329801836578
Pat-JG26		MALE	817	821	23.3598226	FALSE	4	9.7293643823097	9.70796734910994	6.5464411159892	7.15618775950004
Pat-JG28		MALE	700	705	6.7023964	TRUE	2	9.62580279537653	9.84764457558552	6.3463263405505	8.10491711328965
Pat-JG30		MALE	837	841	13.0105341	FALSE	3	10.0156682738079	9.8259477888929	6.14187050873839	6.30552990237904
Pat-JG32		MALE	866	869	15.6717797	FALSE	3	9.47165153685243	10.5643573523785	6.42836936403653	8.01147872810892
Pat-JG34		FEMALE	928	931	34.6947576	FALSE	3b	10.5677266239569	10.6748008437323	6.77977646409272	7.32803477695798
Pat-JG36		FEMALE	819	821	0.0000000	TRUE	2	9.73950218360529	9.07022979227076	5.98788544608275	7.46453323914939
Pat-JG38		MALE	899	902	0.0000000	TRUE	3b	9.69542736716165	10.1401927446724	6.27058078488092	7.23321886432942
Pat-JG40		MALE	912	916	10.6121276	FALSE	1	9.99098230674	10.228844727222	6.18413049755896	7.59130113817375
Pat-JG42		MALE	455	458	8.2465759	FALSE	2	9.91799057277176	10.2069889904269	6.27077559834025	7.1373413612784
Pat-JG44		MALE	798	811	31.5078338	FALSE		10.4687577304294	10.9553231377159	6.6087930095822	8.29148266922621
Pat-JG46		FEMALE	732	733	0.0000000	TRUE	1	9.03973865887085	10.3195374410183	5.94255004694169	7.32480394784175
Pat-JG48		MALE	829	832	23.2612579	FALSE	1	9.53627783683737	9.3458615270087	5.93924485129285	8.754608387631
Pat-JG50		MALE	648	650	23.7869361	FALSE	1	9.21040696205692	9.70931866209768	6.13840681234205	6.98701709308822
Pat-JG52		MALE	929	932	7.7208978	FALSE	4	10.2236445593094	9.96652321665894	6.84932353692797	7.67308101914763
Pat-JG54		FEMALE	685	689	7.3266392	FALSE	2	10.3698435187567	9.43098174197723	6.90294067079801	8.0056090065699
Pat-JG56		MALE	731	735	15.2446662	TRUE	2	8.87767253795456	9.77605155251985	6.05948805740382	7.54560738101239
Pat-JG58		FEMALE	674	677	0.0000000	FALSE	2	10.0747789549313	10.5870451848506	6.51474891052344	7.50615765688944
Pat-JG60		MALE	561	564	22.5384505	FALSE	2	9.72220441411972	10.0061693165844	6.09618345537365	7.83838298054191
Pat-JG62		MALE	749	752	22.2099016	FALSE	4	9.17565784725543	11.0878743984749	6.55933029362852	8.03751114666891
Pat-JG64		MALE	611	615	17.9716216	FALSE	4	8.76784132791897	10.2847470728594	6.40495081407863	7.38728708524353
Pat-JG66		MALE	876	879	0.0000000	TRUE	1	9.57891964331535	10.1165797888942	6.31671534973123	7.04876772525669
Pat-JG68		FEMALE	563	566	0.0000000	TRUE	2	9.99695214236266	9.66126081285082	6.72812776696585	7.14485322286778
Pat-JG70		FEMALE	868	872	16.1974579	FALSE	2	9.3191225204396	10.0612211418024	5.86203634025691	8.30020317892148
Pat-JG72		MALE	461	465	6.3738475	FALSE	2	8.88004739718471	10.27171581355	5.72174079801253	6.96215254950745
Pat-JG74		MALE	902	905	13.0105341	FALSE		10.0797497360588	10.4411512340927	6.22342099668726	8.66431285381999
Pat-JG76		FEMALE	955	960	2.9569396	FALSE	4	9.94515563457634	10.5447373712275	6.44781798971449	8.11600065084322
Pat-JG78		MALE	728	731	13.0433890	FALSE		9.7895887249862	10.901411989869	6.15325942922446	8.0767564270762
Pat-JG80		MALE	912	915	1.7413089	FALSE	2	9.99098230674	9.46307981742183	6.62782406535132	7.49200602207002
Pat-JG82		FEMALE	593	593	12.5834206	FALSE	3	9.19913278891553	10.1917709560967	5.87500438238059	8.09292818790451
Pat-JG84		MALE	596	599	11.5649192	FALSE		9.16991656653822	10.8348374266123	6.2083808683866	8.27596569551153
Pat-JG86		MALE	687	691	9.8236103	FALSE	2	9.41037787302977	10.512563781673	6.02455728660829	8.0289920816864
Pat-JG88		MALE	923	927	8.2794308	FALSE	2	9.31820896773265	10.5328889957611	6.26510362756165	8.56538962354512
Pat-JG90		MALE	753	754	0.0000000	FALSE		10.2109309590427	10.0855215322497	6.14458802391528	6.70900099457215
Pat-JG92		MALE	865	868	22.6698700	TRUE	2	9.64017203627823	10.1296052934051	6.04500330733313	7.39242862059023
Pat-JG94		MALE	705	708	0.0000000	FALSE	2	9.4236289772544	9.71905807702446	6.34480083310527	7.26186810615743

Sheet1

patient	cancer detailed location	gender	age_at_pathologic_diagnosis_by_biopsy_months	age_at_surgery_months	time_to_progression_months	dfs_event	tumor_grade_at_surgery	RAD51 mucosa	RAD51 tumor	BRCA2 mucosa	BRCA2 tumor
Pat-JG96		MALE	913	915	20.1728988	FALSE	2	9.3748653150296	10.5008081443738	6.32571072447587	7.55746965006727
Pat-JG98		MALE	825	828	5.8481694	FALSE	3	9.649334858804	10.0966099180031	6.50393985618687	7.98130671454916
Pat-JG100		MALE	733	736	0.0000000	FALSE		9.44727731092814	10.5373693621352	6.27508820379213	8.44912566519627
Pat-JG102		FEMALE	793	796	0.0000000	FALSE	1	9.61565057818915	9.55204383270225	6.47756460037906	7.74051727603916
Pat-JG104		MALE	783	787	0.0000000	FALSE	4	9.5252132425963	10.3267670373112	6.04014708567165	7.12976625832868
Pat-JG106		MALE	674	675	0.0000000	FALSE		9.7320077351316	10.9093866489973	6.60233222324021	8.82635920474375
Pat-JG108		MALE	864	865	0.0000000	FALSE		9.58040890602739	9.77840626706657	6.03562126310202	7.27333589296663
Pat-JG110		MALE	817	820	0.0000000	FALSE		8.61189023270391	9.332273526541	5.76246523256556	6.61701240615369
Pat-JG112		MALE	942	946	0.0000000	FALSE	4	10.2396538818521	10.4147433658105	5.93257611171002	5.69662980238902
Pat-JG114		MALE	750	789	0.0000000	FALSE		10.203674632942	9.52083719853102	5.7622570078745	5.95608432939918
Pat-JG116		FEMALE	681	684	0.0000000	FALSE	4	10.3308782847836	10.1429080142732	6.92340210504586	7.60216700800857
Pat-JG118		MALE	784	788	0.0000000	FALSE		9.35300814449538	10.4372032983248	6.40339320827231	8.02719169496466
Pat-JG120		MALE	NA	889	29.0108626	TRUE		9.89464424983172	10.0057495477186	6.20733397615604	7.59455504228822
Pat-JG122		FEMALE	653	NA	NA	NA		10.400444512626	10.8863272498094	6.6141427762952	8.90157843019539
Pat-JG124		FEMALE	783	783	11.9920327	FALSE		9.84691882827996	10.1535816806486	6.33895667917076	6.65695991010808
Pat-JG126		FEMALE	609	618	0.0000000	TRUE	1	9.08451766611881	9.79440513115618	6.21500397168323	7.44631815207732
Pat-JG128		MALE	574	577	1.7741637	FALSE	0	10.0855215322497	10.6369624769686	6.58279470387102	7.47742960157217
Pat-JG130		FEMALE	671	675	0.6899526	FALSE	1	9.79351046936302	10.4957846023415	6.4467640696073	7.7297283366043
Pat-JG132	REKTUM	MALE	NA	742	123.8957679	FALSE	4	9.62774383385206	9.80422630581478	6.5540596662742	7.73240264405514
Pat-JG134		MALE	NA	785	90.9423191	FALSE	3a	9.53512842427312	10.3664906054096	6.50958205723249	8.82234524065974
Pat-JG136	REKTUM	MALE	587	590	120.0517464	FALSE	3b	9.11731027091343	10.5906191986498	6.04509075145872	8.80162042491029
Pat-JG138	REKTUM	MALE	760	764	86.3426354	FALSE	3b	9.48162799167322	9.76861171402723	6.35025661090672	8.45695570556547
Pat-JG140	REKTUM	FEMALE	699	702	88.0839442	FALSE	3a	10.6349411023387	10.2721422546332	6.78830800893699	8.53024085631131
Pat-JG142	REKTUM	MALE	NA	796	99.9774123	FALSE	4	9.44384991582416	10.3386894866311	6.23254699149784	8.69201014646551
Pat-JG144	REKTUM	MALE	741	744	3.1540689	FALSE	2	10.1619154769658	10.8536160889588	6.54926039946853	7.93600945865103
Pat-JG146	REKTUM	MALE	684	688	31.7049631	TRUE	2	9.8589728277045	10.2729979361636	6.32916743404762	7.93980902451441
Pat-JG148	REKTUM	FEMALE	NA	580	88.9710261	FALSE	3a	10.0246212337956	10.9485354668893	7.0578823432146	8.50487353703016
Pat-JG150	REKTUM	MALE	850	854	79.4759646	FALSE	3b	9.90872351313217	10.4757010296799	6.48673167002857	8.73082354339933
Pat-JG152	REKTUM	FEMALE	852	854	85.1598595	FALSE	2	9.4557572660463	10.5271331527458	6.54919298812324	8.29354938049326
Pat-JG154	REKTUM	MALE	NA	631	62.8842481	TRUE	1	10.2812300139627	10.5326419955777	6.7657229655633	7.69643954872366
Pat-JG156	REKTUM	MALE	840	843	45.7011438	FALSE	1	10.1240905625931	9.70901103989512	6.63905013550724	8.10397394448937
Pat-JG158	REKTUM	FEMALE	NA	647	79.5088195	FALSE	3b	10.5541980136486	10.5802535407581	6.58445181925029	8.0926197955562
Pat-JG160	REKTUM	MALE	791	794	86.0469414	FALSE	4	9.88905869851933	10.5463201580905	6.53017263947218	7.53681704366412
Pat-JG162		MALE	641	644	58.8759523	TRUE	2	8.96236378099289	9.98368260105678	6.02167157648896	6.80209756231847
Pat-JG164	REKTUM	MALE	NA	720	60.0258732	FALSE	3a	9.31864733650426	10.2421110056718	6.17731778417097	8.25465600389115
Pat-JG166	REKTUM	MALE	815	818	61.4057784	FALSE	1	9.93984567010743	10.1107795486369	6.37137866346891	8.13537514450566
Pat-JG168	REKTUM	MALE	783	788	25.3639705	TRUE	3b	10.0599037676567	11.5651574279308	6.85445775520778	8.05038462691463
Pat-JG170	REKTUM	MALE	952	956	71.8864864	FALSE	2	9.932600675459	10.5897480507154	6.02009257983227	7.70606773722108
Pat-JG172	REKTUM	FEMALE	NA	976	60.7486807	FALSE	3a	9.84592811485597	10.6059164624116	6.08811257866425	7.71221000621632
Pat-JG174	REKTUM	MALE	793	796	71.2622436	FALSE	3b	9.31891985865312	10.2040209364471	6.02508494832312	8.10155366708474
Pat-JG176	REKTUM	MALE	NA	711	61.2086490	FALSE	3b	9.35827400930955	9.64054058714024	6.09307120549181	7.77504602318562
Pat-JG178	REKTUM	MALE	NA	688	71.9521961	FALSE	3b	10.3497918589499	10.4210851146152	6.27046952026899	7.9349526973149
Pat-JG180	REKTUM	MALE	NA	978	39.2287316	FALSE	3b	9.4561269657962	10.4291463381227	5.41427943536627	8.07512500763003
Pat-JG182	REKTUM	MALE	NA	748	34.3662088	FALSE	1	9.72093472254815	10.2724811946416	6.27909816248571	7.19881746886859
Pat-JG184	REKTUM	FEMALE	NA	763	88.7738968	FALSE	2	10.2295043265869	10.3909610789031	6.91452686826201	7.49079334207042
Pat-JG186	REKTUM	FEMALE	798	802	79.5745293	FALSE	4	10.4411512340927	10.6298253335457	6.89044455629873	7.5205975409638
Pat-JG188	REKTUM	FEMALE	854	858	73.4306659	TRUE	2	9.91550729755446	10.1911953095551	6.44268656032101	7.85907810027254
Pat-JG190	REKTUM	MALE	918	921	77.8003655	FALSE	3	9.9070276439687	10.4512905046282	6.64469478259682	8.31092175035439
Pat-JG192	REKTUM	FEMALE	914	918	75.4348139	FALSE	4	9.70530790418866	9.45321793703465	6.49472592247793	8.07828547537225
Pat-JG194	REKTUM	FEMALE	695	700	72.6750036	FALSE	3a	9.96086886079383	9.76662157164794	6.66984339639354	7.83921768968108
Pat-JG196	REKTUM	MALE	918	920	80.2316269	FALSE	1	10.522376601178	9.64270663690292	6.62507547401101	8.00690778590952

Pat-JG198	REKTUM	FEMALE	NA	750	34.2676441	TRUE	3	9.32698543428459	10.1826968548391	6.20921452097672	7.94928840877884
Pat-JG200	REKTUM	MALE	610	614	76.8147190	FALSE	2	10.854316245525	10.5825865201614	6.93176246016122	7.97786723093612
Pat-JG202	REKTUM	FEMALE	810	813	29.7993799	FALSE	2	9.64355425827559	10.3262977589806	6.30091548435709	7.69047666179671
Pat-JG204	REKTUM	MALE	NA	760	64.6584119	FALSE	3	9.27161022573418	9.0192253653749	5.98205165249095	7.95181049404982
Pat-JG206	REKTUM	FEMALE	912	916	71.5579375	FALSE	3b	10.5911242395901	10.90925648309	6.87753136441826	8.27603876082734
Pat-JG208	REKTUM	FEMALE	503	NA	NA	NA	3b	10.7087008159918	11.1696408809806	7.08010958566877	8.13883084441157
Pat-JG210	REKTUM	MALE	NA	696	59.9930183	FALSE	1	10.6081459876654	10.2275520456921	7.07678302657331	7.95689965585809
Pat-JG212	REKTUM	MALE	NA	789	60.7486807	FALSE	3b	8.60010915424572	9.75670403301705	6.61681560358252	8.1839155427794
Pat-JG214	REKTUM	FEMALE	714	NA	NA	NA	4	10.4535732470266	8.62656129494514	6.08242345449241	7.2595586564002
Pat-JG216	REKTUM	MALE	640	643	27.1052794	TRUE	3b	10.4734142702646	10.6156906815573	6.66054851991928	7.53261639464711
Pat-JG218	REKTUM	MALE	NA	682	60.3215672	FALSE	4	9.88837265627446	11.0262588572588	6.64975033333928	7.7140565700071
Pat-JG220	REKTUM	MALE	725	728	57.7917411	FALSE	4	10.4510419331833	10.709316241893	6.68715810993484	7.39616767850495
Pat-JG222	REKTUM	FEMALE	767	768	47.2453233	TRUE	2	9.54905988191997	9.65970133328911	6.41800874323664	6.87606102333389
Pat-JG224	REKTUM	MALE	662	665	0.0000000	TRUE	3a	9.8471287269081	9.78624631194807	6.6160778132491	7.31022588120203
Pat-JG226	REKTUM	MALE	596	600	12.6162755	TRUE	1	10.3267670373112	10.4855709199509	6.10002668711922	7.23801340671052
Pat-JG228	REKTUM	MALE	NA	427	28.9122980	TRUE	1	10.0114070516876	10.6829733276843	6.5215133634938	7.72730684876835
Pat-JG230	REKTUM	MALE	930	933	71.9521961	FALSE	2	10.2204830535039	10.6070184607782	6.73461231039549	7.2676017140131
Pat-JG232	REKTUM	MALE	777	781	59.6973244	FALSE	2	10.0973023910621	10.1891679638454	6.81358030785512	8.54847802013938
Pat-JG234	REKTUM	FEMALE	892	895	18.6287193	TRUE	3b	9.78634147862271	10.5867883319483	6.67692344610538	7.30157435249666
Pat-JG236	REKTUM	MALE	752	756	62.2271505	FALSE	3b	9.66322096608765	10.5754602445943	6.59321193574483	8.09744869763277
Pat-JG238	REKTUM	MALE	875	879	60.7158258	FALSE	3b	9.20911244788602	10.7675865094839	6.5887509350207	7.48813852293009
Pat-JG240	REKTUM	MALE	NA	879	59.4673402	FALSE	3b	9.78836062691856	9.9111042216155	6.85581210810502	7.87520251547377
Pat-JG242	REKTUM	MALE	NA	657	33.0848683	TRUE	3b	10.0699016799775	10.3258311302058	6.69115331863707	7.8148956085
Pat-JG244	REKTUM	MALE	733	738	2.3984065	FALSE	3a	9.06641212904018	10.5471942769553	5.99384716193497	8.77379500725143
Pat-JG246	REKTUM	FEMALE	755	758	14.9818271	TRUE	3b	9.52941352663323	10.7957387019814	6.45191637267061	8.45827858869724
Pat-JG248	REKTUM	MALE	763	767	23.0312737	FALSE	2	10.7773014270062	9.98611211440116	6.96597251245161	8.25813604840777
Pat-JG250	REKTUM	MALE	901	905	12.8134048	TRUE	3b	9.86203587149641	9.46607612471635	6.6849801163812	7.20826137185643
Pat-JG252	REKTUM	FEMALE	815	819	47.8367112	FALSE	4	10.1049230442141	10.2216130623954	6.80886091046357	7.79764237108892
Pat-JG254	REKTUM	FEMALE	609	613	18.1687509	TRUE	1	9.88418031354158	10.8479731559824	6.52667352390912	8.2850449225161
Pat-JG256	REKTUM	FEMALE	552	555	48.4609540	FALSE	4	9.6165734450523	10.9397830135036	6.33841579250567	7.13964011856864
Pat-JG258	REKTUM	FEMALE	NA	713	63.6727654	FALSE	3b	9.85303713614205	10.2466735094762	6.27137977602105	8.37288247464671
Pat-JG260	REKTUM	FEMALE	847	850	33.1177232	TRUE	3b	9.4527026484896	10.2320928428814	6.45685834644327	7.1949118877766
Pat-JG262	REKTUM	MALE	714	717	60.2230025	FALSE	2	10.1801049676744	11.4604779116467	6.48718127106043	7.62311603089404
Pat-JG264	REKTUM	MALE	970	973	44.5840777	FALSE	2	10.0372728073666	11.5837249767879	6.93264777701362	8.32267199270536
Pat-JG266	REKTUM	FEMALE	871	875	46.9167745	FALSE	3b	10.144021596241	10.2424858802196	6.56327345495345	6.88393422850719
Pat-JG268	REKTUM	MALE	846	850	59.7301793	FALSE	2	9.43147115210448	9.6704238255805	6.26479532022127	6.9725570318282
Pat-JG270	REKTUM	MALE	NA	766	24.9697119	TRUE	2	9.69232416897864	10.2624985272802	6.40266293398973	7.28788081458395
Pat-JG272	REKTUM	MALE	760	764	59.7958890	FALSE	3	9.93814493970538	9.90568829802604	6.33783447617133	8.25584940377644
Pat-JG274	REKTUM	MALE	863	867	0.0000000	TRUE	3b	9.85727788909805	10.0800843723662	6.4152991166405	7.40154128431424
Pat-JG276	REKTUM	MALE	619	622	0.0000000	TRUE	1	9.97486422089726	9.98431256293956	6.66513384503108	7.2660074374623
Pat-JG278	REKTUM	FEMALE	830	834	59.7630341	FALSE	4	9.7673333284798	10.9664699931108	6.149462534978	8.11600065084322
Pat-JG280	REKTUM	FEMALE	778	781	48.4938089	FALSE	2	10.0507851454335	10.9313256776961	6.57078067999794	8.49407746405616
Pat-JG282	BLASE	MALE	NA	NA	NA	NA		9.53429623966274	10.132904040434	6.25886983805001	7.48101302456527
Pat-JG284	REKTUM	MALE	NA	722	73.7920697	FALSE	4	10.1287058374028	10.7521018262584	6.54588164000858	8.25813604840777
Pat-JG286	REKTUM	MALE	655	658	4.8296680	TRUE	2	9.76611938630267	9.10936828893964	6.25403457256147	6.16745096821405
Pat-JG288	REKTUM	FEMALE	672	675	47.8038563	FALSE	1	10.0661818019134	10.708582228281	6.37226227175836	7.47945594012763
Pat-JG290	REKTUM	MALE	792	794	41.1343149	FALSE	2	9.72903174602245	9.98249975238925	6.21035466608885	7.9277765841596
Pat-JG292	REKTUM	MALE	955	958	47.5738722	FALSE	3b	9.57870091382236	9.91221482158525	6.06953338398285	7.52023717068601
Pat-JG294	REKTUM	MALE	806	810	54.6376722	FALSE	2	10.3576831527751	9.53409451419075	6.89104908355076	7.50268320196662
Pat-JG296	REKTUM	MALE	NA	726	63.0485226	FALSE	1	9.89658767938282	11.3462158829689	6.51795872620206	8.66243915914903
Pat-JG298	REKTUM	FEMALE	617	621	71.1965338	FALSE	2	9.78894788907853	10.6204278596121	6.57336439973531	8.64673315967772

Pat-JG300	REKTUM	MALE	NA	689	0.0000000	TRUE	2	10.215784148324	9.99917307241859	6.5448179502763	7.2035716173109
Pat-JG302	REKTUM	MALE	652	657	52.4035401	FALSE	3b	9.7689475787815	8.98664623506857	6.56366602786653	7.67999196926111
Pat-JG304	REKTUM	FEMALE	880	884	25.7910840	TRUE	2	10.7136906759326	10.0471488010061	6.58057932434449	8.037809983039
Pat-JG306	REKTUM	MALE	740	743	47.8367112	FALSE	3	9.89576601791338	10.4659182124574	5.98083903680363	7.92704466644866
Pat-JG308	REKTUM	FEMALE	883	887	22.9984189	TRUE	2	9.4403012244641	10.8415616066723	6.5652954813932	7.95138011359036
Pat-JG310	REKTUM	FEMALE	603	606	35.8775334	FALSE	2	10.3153054148497	11.103676096261	6.45573750262466	7.42300963187402
Pat-JG312	REKTUM	FEMALE	NA	923	54.9990760	FALSE	2	9.61985795401814	9.51637545681719	6.68618527040748	7.96205929295081
Pat-JG314	REKTUM	FEMALE	NA	501	36.1075176	FALSE	3b	10.7392351934426	10.6526532648296	7.21648310084744	8.88071092100141
Pat-JG316	REKTUM	MALE	NA	876	33.8076757	FALSE	4	9.57859965160822	10.813394394519	6.10616749250596	7.60216700800857
Pat-JG318	REKTUM	MALE	496	499	0.0000000	TRUE	3a	9.63899585193679	10.648286056648	6.5689637202148	8.12454971510677
Pat-JG320	REKTUM	MALE	799	804	37.2245837	FALSE	4	10.0313817874561	10.0651935143657	6.48058334129658	7.93147420831018
Pat-JG322	REKTUM	MALE	738	743	55.4261894	FALSE	4	10.1002527436542	10.2035676789026	6.34342505643478	7.08233007862914
Pat-JG324	REKTUM	MALE	499	504	46.4568061	FALSE	4	10.0027907637881	8.9194432717379	6.74017031334999	7.32621684611688
Pat-JG326	REKTUM	MALE	819	824	36.0418078	FALSE	3b	9.7139350330664	9.85845906892524	6.25673065116432	7.43985505965958
Pat-JG328	REKTUM	FEMALE	708	712	0.0000000	TRUE	3b	10.8512258815258	10.421324753968	7.13594328754322	7.41604257583028
Pat-JG330	REKTUM	MALE	813	816	0.0000000	TRUE	2	9.16616257553876	9.71446410813288	5.43986390096236	7.7683117913714
Pat-JG332	REKTUM	FEMALE	941	945	37.4545679	FALSE	3	9.63661912068068	9.82026591275	5.68509648556019	7.12001670454561
Pat-JG334	REKTUM	MALE	684	687	35.8775334	FALSE	4	9.27375230382975	10.556789843747	6.15202814208141	7.37231050379047
Pat-JG336	REKTUM	FEMALE	762	764	38.3745046	FALSE	3b	9.72998580323565	11.9170575672389	6.28721138198921	7.76809472022785
Pat-JG338	REKTUM	MALE	725	728	27.3024087	TRUE	3a	9.88837265627446	10.6842920523487	6.40060310576944	8.67908522577772
Pat-JG340	REKTUM	MALE	860	864	18.6287193	TRUE	2	9.92319243587035	9.87132422203354	5.2205282589978	6.45658047445312
Pat-JG342	REKTUM	MALE	746	750	35.8775334	FALSE	3b	9.9474572302721	10.1870499100759	6.4831773398454	6.47966600031446
Pat-JG344	REKTUM	FEMALE	617	620	21.3885295	FALSE	3b	10.5195752116935	10.2674192594574	7.55218340983208	7.43976945508425
Pat-JG346	REKTUM	FEMALE	674	678	34.1690794	FALSE	2	10.0496002930665	10.2527036880592	6.17513316012296	6.77570936196489
Pat-JG348	REKTUM	MALE	650	NA	NA	NA		9.35131075560352	10.1635408859291	6.13855475409213	7.53023660210414
Pat-JG350	REKTUM	MALE	NA	899	2.2012772	FALSE	2	10.314108587286	9.75274652333229	6.59493067652669	6.96336453093771
Pat-JG352	REKTUM	MALE	736	738	23.6555165	FALSE	1	9.50418216243458	10.3714065116273	5.76552482941646	7.35932005743421
Pat-JG354	REKTUM	MALE	833	838	34.1690794	FALSE	3b	10.332296636157	10.3463498043445	6.36817080867901	7.22132675100658
Pat-JG356	REKTUM	FEMALE	758	763	36.3375018	FALSE	3b	10.0218374139692	11.0991228561188	5.81370128253054	8.27244205352121
Pat-JG358	REKTUM	FEMALE	703	707	8.4437052	TRUE	2	9.868789562673	9.12976876175697	6.24878110921318	5.98205165249095
Pat-JG360	REKTUM	FEMALE	634	637	36.5674860	FALSE	4	9.05069185629507	10.6467036009497	6.07223954217169	8.31917030340053
Pat-JG362	REKTUM	MALE	779	783	24.9040021	FALSE	3a	9.14851626384365	10.0916112300662	6.39416402700528	6.46858029637206