Supplement S3: Evaluation studies for *in silico* prediction tools

	Evaluation data	Align-GVGD			SIFT			MutationTaster2			PolyPhen-2			Remarks
		SENS	SPEC	ACC	SENS	SPEC	ACC	SENS	SPEC	ACC	SENS	SPEC	ACC	
Rodrigoues et al., 2015 [1]	48 variants in *UGT1A1*	0.65	0.67	0.65	0.92	0.80	0.80				0.89	0.40	0.75	with manually curated input alignment of 27 *UGT1A1* orthologs
Mueller et al., 2015 [2]	339 variants in 76 genes				0.56	0.54	0.55				0.48	0.69	0.60	
Luxembourg et al., 2014 [3]	52 variants in *SERPINC1*				0.80	0.67	0.79				0.94	1.00	0.94	
Grimm et al., 2015 [4]	5 data sets with numbers of variants in range from 8850 to 47149				0.62–0.79	0.62–0.80	0.64–0.79	0.74–0.93	0.51 –0.85	0.60–0.88	0.65–0.83	0.62–0.81	0.63–0.82	
Choi et al., 2012 [5]	57646 human missense variants from UniProt				0.85	0.69	0.77*				0.89	0.62	0.76*	*balanced accuracy
Leong et al., 2015 [6]	312 variants in *KCNQ1*, *KCNH2* and *SCN5A*				0.82	0.77	0.81				0.86	0.40	0.81	
Hicks et al., 2011 [7]	33 variants in *BRCA1*, 30 variants in *MSH2*, 60 variants in *MLH1*, and 144 variants in *TP53*	0.00–0.97	0.52–1.00		0.32-0.95	0.18–1.00					0.67–0.97	0.18–1.00		
Miosge et al., 2015 [8]	30 variants in 23 mouse immune genes				1.00	0.58	0.63				1.00	0.42	0.50	nsSNPS were confirmed via *in vivo* tests for loss of function phenotypes
Kerr et al., 2017 [9]	1118 missense variants from the Myriad Genetic Labratories, Inc. database	0.84	0.92	0.91	0.99	0.56	0.60				0.81	0.70	0.71	750 variants were excluded from Align-GVGD evaluation as they were already used for its training

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