

a

Region-1

D29	1	-----M-----SKP
SWU1	1	-----M-----SKP
Echild	1	MSLQLG-----SNGDLVNRWIRVMKARFASYA-----GKLRED---AYFGLDDQAVQREYETRTHQ-----TPDGIVTDGDLAY--LLP-----AKP
RedRock	1	MSLKVG-----SSGEVVSRWIRVMKARFVSYA-----GKLAVD---GYFGLDDAEVQREYERRTGQ-----TPDGIVTDGDLAY--LLP-----RKP
Goose	1	MALKVG-----SSGPLVNAWIRTMNARFESYSKAYDGGPLRED---AYFGYDDAEVQREYERRTGQ-----PVDGEVSDHDLVALGLIT-----EKP
Twister	1	MPLKLG-----SSGELVNAWIRTMNSRFESYSKAYDGGPLRED---AYFGYDDAEVQREYERRTGQ-----PVDGEVSDHDLVALGLIT-----EKP
Sheen	1	-----MTR
Timshel	1	MILKVG-----STGQVVDWQAEMLERYPYAKAADGGPLKVD---GYFGYDDEAVQVEYEFRTGQ-----PIDREVSQGDLDYLGIKTDS---FKGR
Heldan	1	-----MSDR
BabyRay	1	-----MSDR
BTCU-1	1	-----MSDR
Anubis	1	MPLRVGSND-----ANTGGLVSRWQKTMLARFQAYAKAYDGGPLRVD---GYFGYDDADVQREYERRTHQ-----TVDGEVSDADLRALGLEA-----AKR
Bxz2	1	MPLRVGSND-----ANTGGLVSRWQKTMLARYAAAYAKAYDGGPLRVD---GYFGYDDADVQREYERRTHQ-----VVDGEVSDADLRALGLEA-----AKR
Severus	1	MPLRLG-----DRNDTVREWRYIMSKRFSGYARALGPLPT-DT---NEFGPRAKSWQEEYERRTGQ-----PVDGEVSDLDLFDALGVSVSHPAPGPTR
Zakai	1	--MAVGPDGKWIGYGLGDSGIPVLTARQKLKAKF-SYAKH-----LRET---TLYDNELLPVLEIYQLRKNVEFAKAGKKLLRTDGILDYATQVAIGVVI--PVALVKP
Wildcat	1	----MRVAGQWVGWGLGDIDPKVQDMKRFLKRKF-SYARES-----LDDS---EYDEMTMRVVMQMANY-----GDLDITGVMNYATQVRSGYLKVEK--PPLP
Ardmore	1	----MRIDGQYVGLGPGDRSDEIRKIKAFMRKKF-SYAATLA---DT-----EFYDEAMTAVVAEMQSRYNT--AGHLRDGLYIPGIINAETKYVMGYLSRPV-IDTRP
Ms6	1	----MRIDGQYVGLGPGDSSEEIRRIKTFMRKKFASYAGDLA---DT-----PLYDEQMTAAVAEMQARYNT--AGQLRDGLYIPGIINAETKYVMGYLSRPV-IDTRP
Hades	1	----MRIDGQYVGLGPGDSSEEIRRIKTFMRKKFASYAGDLA---DT-----PLYDEQMTAAVAEMQARYNT--AGQLASGLYIPGIVGAETKYVMGYLPRPV-VDTRP
Saal	1	----MRIDGQYVGLGGLGDSDEIRKIKAFMRKKFASYAGDLA---DT-----PLYDEAMTAAVAEMQARYSA--AGQLRAGLYIPGIVGAETKYVMGYLPRPV-VDTRP
Pumpkin	1	-----MTTYGELKALRLG---VKYVRH
Graduation	1	-----MPLKLGDRNPTVRRWREVMAARFAGYARVHGPLPTDTDEFGP-----RAEAWQTEYE-----SRTFQPLDGIVSDDD---LRALGIPAPEDTRP
MrMagoo	1	-----MAWIGWQEGMEGLPVLAAGELRAKF-SYGKQ---LELT---QKFTPELTQAIKTFQVKKG-----GLRTDGVLDYATQAALGVPE-----ALKP
Dylan	1	----MRIGGEYVGLGLGDASEEIGRIRDFMRKKFRSYAGNLP---DTRNALGLPLFDEPMTVAVTEMQGRYLQ--SGELRDGLFIFGIINAETKYVMGYLERPPGPDTRP
DS6A	1	-----MTWIGWQQGMAGEPVAAAKRELRRKF-SYAKH-----LDDS---DVFDLELLAVLIEYQQKKNAT---GYTPRLREDGVLDWATQVAIGTVQTGP-PPKAG
Palestino	1	-----MLKLGSSGLMVSAAWTAVMRLRFASYALGVNGQPIKVD---GYFGYDEEKVQKEYQLRTGQ-----FPSGQVSREDLHRLGLLP-----
Obama12	1	-----MLKLGSNGLMVAAWTAVMIRIRFASYALGVNGLPIKVD---GYFGYDEEKVQKEYQRRTGQ-----FPSGQVSREDLHRLGLLP-----
Omega	1	-----MSLWPEIR---AMLNGEFYVPAG
Larva	1	-----MSKP
Enkosi	1	-----MSKP

b

Accession	Position	Sequence	Reference
D29	5	WLFTVHGTGQPDPLGPGLPADTARDVL----	DIYRWQPIGN-YPAA----
SWU1	5	WLFTVHGTGQPDPLGPGLPADTARDVL----	DIYRWQPIGN-YPAA----
Echild	73	WLFTVHGTGMPDPLGPGLPADVARDVL----	DIYRWQPIGN-YPAA----
RedRock	73	WLFTVHGTGMPDPTGPGLPADVARDVL----	DIYNWQPIGN-YPAA----
Goose	80	WLFTVHGTGAQPDPLGPGLPADTAKQVL----	DLYRWQPIGN-YPAK----
Twister	80	WLFTVHGTGAQPDPLGPGLPADTAKQVL----	DLYRWQPIGN-YPAK----
Sheen	4	WLYTVHGTGMPDPFGPGLPADTAREVL----	DLYTWQPIGN-YPAA----
Timshel	83	WLFTVHGTGMADPFGPGLPADTARDVE----	DIYDWQPIGN-YPAA----
Heldan	5	WLFTVHGTGQPDPLGPGLPADTARQVL----	DLYRWQPIGN-YPAS----
BabyRay	5	WLFTVHGTGQPDPLGPGLPADTARQVL----	DLYRWQPIGN-YPAS----
BTCU-1	5	WLFTVHGTGQPDPLGPGLPADTARQVL----	DLYRWQPIGN-YPAS----
Anubis	84	WLFTVHGTGMPDPLGPGLPADTARAVL----	DKYTWQPIGN-YPAA----
Bxz2	84	WLFTVHGTGQADPLGPGLPADTARAVL----	DKYTWQPIGN-YPAR----
Severus	85	WLFTVHGTGMADPLGPGLPADTARACL----	DKYTWQPIGN-YPAS----
Zakai	97	LLFTVHGTGQFPVDPGPGLPADTARAVL----	DKWEWQPIGN-YPAD----
Wildcat	87	TLYTVHGTGVSMD--GPPADCARRLL----	DKYRWQPVG-YPAS----
Ardmore	94	VLFTVCGTGVPWWV--GPDADTARAVE----	DQYLWQPIGY--PAA----
Ms6	94	VLFTVCGTGVPWWV--GPDADTARAVE----	DQYLWQPIGY--PAA----
Hades	95	VLITVCGTGVPWWI--GPDADTARAVE----	DKYLWQPIGY--PAA----
Saal	95	VLITVCGTGVPWWI--GPDADTARAVE----	DKYLWQPIGY--PAA----
Pumpkin	20	TLFTVAGTADWMS--GYPADVARLVD----	EDLFRWQPVW--YPASFGPVGNPLGRSYQESVQDGVKELI-----
Graduation	82	VLLTVSGTGVPWWI--GPDADVARRLG----	DVYLWRPVGPPYTAQ----
MrMagoo	79	WVFTVAGTGAGWD--AGYPADVARAVL----	DLFRWWGI-V-YPAK----
Dylan	102	LFITVCGTGVPWWV--GPDADTARACE----	DKLFWQPIGY--PAK----
DS6A	89	TLFTVQGTGVDMMT--GPPADTARAVE----	GDWEWQPIGN-YPAS----
Palestino	76	TLLSIHGTGQADPFGIGYPADIARRVL----	DLYWWQPVGN-WPAK----
Obama12	76	TLISIHGTGQADPFGIGYPADIARRVL----	DLYWWQPLGN-YPAK----
Omega	21	TIITANGTGVPDAGPGFADLARELVAEGMWWKWIN--	YPAA----
Larva	5	MLLTASGTGADMWT--GYPADLARRV----	EDLYYFQPIY--YGPNGIPATVPMQGSQSGVDMGVSLVLQAE--
Enkosi	5	VLLTASGTGADMWS--GYPADLARRV----	EDLYYFQPIY--YGPNGIPATVPMGASQSGVDMGVRLVLECE--

c

			$\alpha 4$	$\beta 4$	Loop-2	$\alpha 5$	$\beta 5$	Loop-3	$\alpha 6$	L-4	$\alpha 7$	$\alpha 8$	
D29	100	TGRLHRRF--LHRLKKVIFW	GNP	MRQKGFAHSDEWIHPVAAPDTL	----	GILE-DRLENLE	----	QYGFVFRDYAHD	----	GD	MYASIKED	----	DMHEYEVA
SWU1	100	RGR LHRRF--LHRLRKVIFW	GNP	MRQKGFAHTDEWIHPVAASDTM	----	GILE-DRLENLE	----	QYSFVFRDYAHD	----	GD	MYASIKED	----	DMHEYEVA
Echild	167	KGRLHKY--VNRLKKVVMW	GNP	MRQKGFAHFDEWIHPVAAPDTM	----	GILE-DRLENLEQA-MQ	QYGFVFRDYAHD	----	GD	MYASIKED	----	DMHEYEVA	
RedRock	167	KGRLHKY--VHRLKKVVLW	GNP	MRQKGFAHFDEWIHPVAGPDTM	----	GILE-DRLEALEQA-MK	VYGFVFRDYAHD	----	GD	MYASIKED	----	DMHEYEVA	
Goose	168	KGRLHKY--LHRLKKVVFV	GNP	MRQKGIAHFDEWIHPVAGPDTM	----	GILE-DRLENLE	----	QYDFVFRDYAHD	----	GD	MYASIKED	----	DMHEYQVA
Twister	168	NGRLHKF--LHRLKKVVFV	GNP	MRQKGIAHFDEWIHPVAGPDTM	----	GILE-DRLENLE	----	QYDFVFRDYAHD	----	GD	MYASIKED	----	DMHEYQVA
Sheen	94	TGRLHHR--LGDVRKVVFV	GNP	MRQQGIAHSDEWVHPVASPDTM	----	GILE-DRLEGLE	----	NAPFVFRDYAHQ	----	GD	MYASIKAD	----	DMHEYQVA
Timshel	173	SGSLHHR--LKDVRKVVFV	GNP	MRQQGIAHTDEWIHPVASPTM	----	GILE-DRLEGLE	----	DAPFVFRDYAHE	----	GD	MYASIKAD	----	DMHEYEVA
Heldan	97	AGEFHYL--LHQVRKVVFV	GNP	MRQRGIAHDDRWIHRIAAPDTH	----	GIME-DRLEGLE	----	HAPFVFRDYAHD	----	ED	MYASIRDD	----	DMHEYQIA
BabyRay	97	AGEFHYL--LHQVRKVVFV	GNP	MRQQGIAHDDRWIHRIAAPDTH	----	GIME-DRLEGLE	----	HAPFVFRDYAHD	----	ED	MYTSIRDD	----	DMHEYQIA
BTCU-1	97	AGEFHYL--LHQVRKVVFV	GNP	MRQRGIAHDDRWIHRIAAPDTH	----	GIME-DRLEGLE	----	HAPFVFRDYAHD	----	ED	MYASIRDD	----	DMHEYQIA
Anubis	174	KGSLHHR--LGDVRKVVMW	GNP	MRQKGIAHDDRWIHKVAGPDY	----	GILE-DRLEGLD	----	KAPFVFRDYAHE	----	DD	MFASVRDD	----	DIHEYQIA
Bxz2	174	KGSLHHR--LGDVRKVVLW	GNP	MRQRGIAHFDEWIHPVAGPDSY	----	GILD-DRLEGLE	----	KAPFEIRDYAHA	----	GD	MYASITDG	----	DKDEYKIA
Severus	175	AGPLHHR--LGDVRKVVFV	GNP	MRQQGIQHDDQWIHEIAPADTH	----	GILTFDLLEGLE	----	RFPHIQVRDYAHK	----	GD	MYACNTDD	----	QAAEYKRA
Zakai	192	GGEFHDQYEAGQVLAVVTW	GNP	MRERGVQHGDGWKE-TAPADTE	----	GILE-DRLEGTP	----	AFWREYAHK	----	GD	MYAACELTG	----	VQRGDNKRA
Wildcat	175	MGR LHHR--LPDFMGGVTW	GNP	MREMGKAWTDGV-GAVAGQNG	----	GIAE-DRLVGTP	----	WNWRDYAHK	----	GD	LYTDCEFD	----	DEGEYKRS
Ardmore	186	DGSLRWM--KPHVRKAVTW	GNP	NRELGHVWADHGGSPMAPSNTQ	----	GVSS-N	-----	G-MRNTPDWWRDYAHQ	----	GD	LYACTEPG	----	DTQEV RNA
Ms6	186	DGSLRWM--KPHVRKAVTW	GNP	NRELGHVWADHGGSPMAPSNTQ	----	GVSS-N	-----	G-MRNTPDWWRDYAHQ	----	GD	LYACTEPG	----	DTQEV RNA
Hades	187	TGSLHWM--KPHIEKAVTW	GNP	NRELGHVWADHGGSPMAPSNTQ	----	GVSS-N	-----	G-MRDTPPWWRDYAHQ	----	GD	LYACTEPG	----	DTQEV RNA
Saal	187	DGSLHWM--KPHIEKAVTW	GNP	NRELGHVWADHGGSPRAPSNTQ	----	GVSS-N	-----	G-MRDTPPWWRDYAHQ	----	GD	LYACTEPG	----	KSGEIITS
Pumpkin	113	FGSLRHR--LKDFIGGGCF	GNP	YRAKGVSYPGSGL----PTSGH	----	GIAP-VNLAPDI	----	LPAEMWEEWNE	----	GD	LYAQNLDG	----	DSGEMKTA
Graduation	176	TGRLHWV--KDHVRGAVTF	GNP	MRETGKVWPNPGGQL-PSAKSH	----	GIAD-Q	-----	L-MVDTPDWWRNYAHK	----	GD	LYTDCE-G	-----	DSGEMKTA
MrMagoo	173	--DL-----EKRIIGSITF	GNP	CRELGVANGNVAE-GIPIEGR	----	GIGD-DRLQSTP	-----	EWYDFAHGANSFPGRDI	YTDTPDD	----	DTGEMMTA		
Dylan	200	NGSLHWA--KDYVVKAVTW	GNP	NREVGAVWPDYGGSPMASLTSQ	----	GVSS-T	-----	G-MRDTPSWWRNYAHA	----	GD	LYAAAEFG	----	DSQQDKNA
DS6A	182	GGMLHDL--LPQVAAGVTW	GNP	MAEKGFHGNRFAG-WPVPAGM	----	GINR-DRLENT	-----	ALWLDFGHGRNSPWGD	DIYCDRPN	----	LAGQDMEL		
Palestino	169	----RRKELRGELVAAASF	GNP	MRLRGHYAGNVD-----PGGE	----	GIDPRQELAAE	-----	PFRVELAAK	----	GD	LYTTCPPG	----	QSGEMERA
Obama12	165	----RRKELRGELVAAASF	GNP	MRLRGHYAGNVD-----PGGE	----	GIDPRQELAAE	-----	PFRIELAAK	----	GD	LYTTCPPG	----	QSGEMERA
Omega	110	DGELHDR--LDDIVAVILY	GNP	VRAPGIAYGNELGGQSAPGKLNHVTGGIAGPDCLRPEECVHPVTGRRIVLDFANA	----	GD	LYAAPVGAEPWVKETEVGHNETL						
Larva	101	TGRLKSV--GHKLMAGSTF	GNP	WRELDAD-----GGR-----GISD-RRI---A-----GTPDFWVDEFDP	----	GD	IYANVPNN-----DVGADMTA						
Enkosi	101	TGRLRHL--NGKLMAGSTF	GNP	WRELDSDN-----GGR-----GISD-RRI---V-----GTPDFWVDEFDP	----	GD	IYANVPNN-----DVGDDMTA						
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d

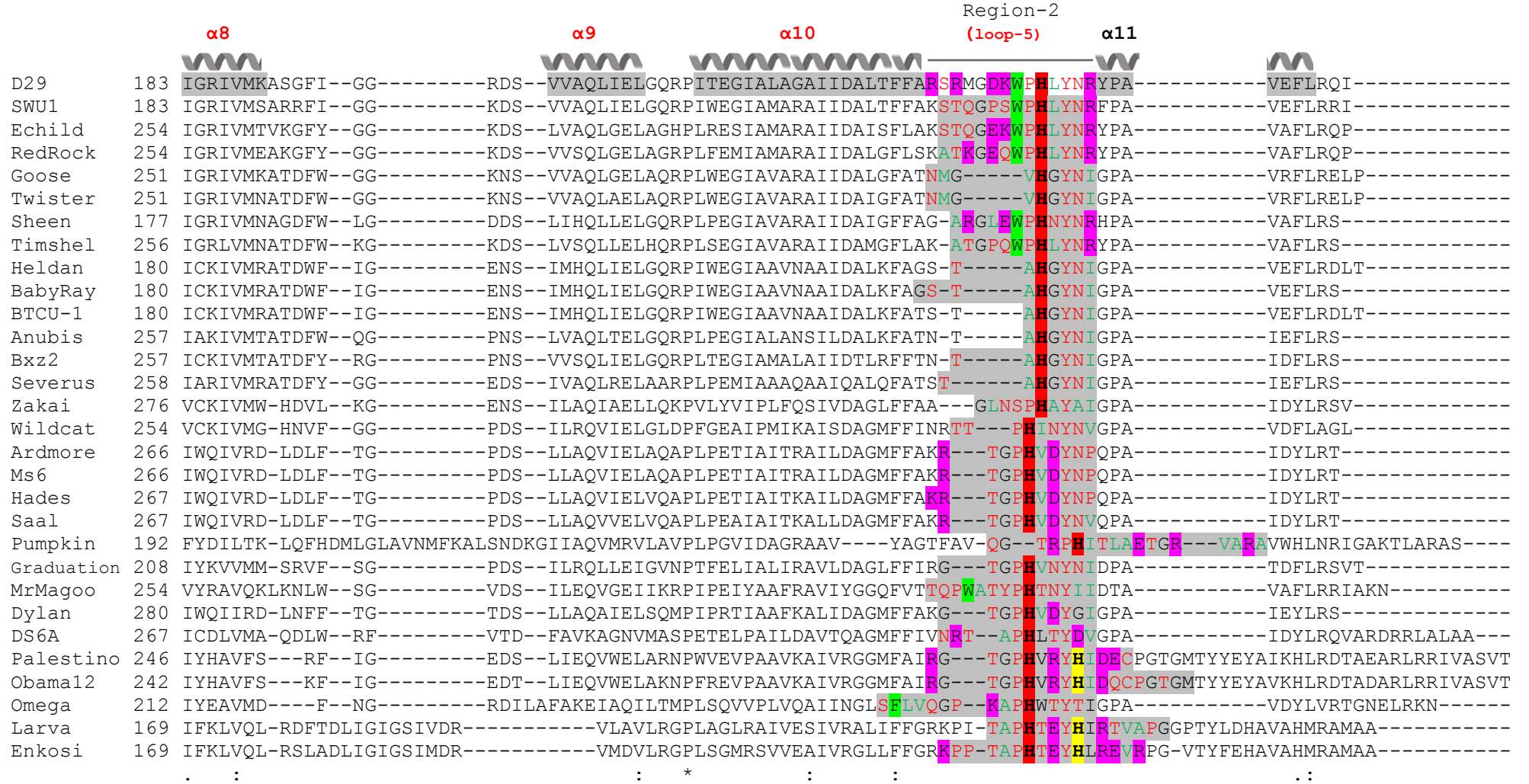


Figure S3: Multiple sequence alignment of LysB-D29 with its homologous lysB proteins. Identical residues are labeled by Asterisk, conserved residues labeled by colon and full stop indicates semi-conserved residues. Residues forming the active site are shaded in red color. Extra His residues are highlighted in yellow color. Oxyanion hole and GXP residues are shaded in pink. Residues forming β -strands and α -helices are highlighted in gray. Region-1 constitutes extra N-terminal residues. Region-2 constitutes residues forming hypervariable loop-5. Loops, β -strands and α -helices forming linker domain are in red. Residues forming loop-5 in each LysB model are highlighted in gray. Polar non-charged residues are in red, polar charged residues are highlighted in pink. Non-polar aliphatic residues are in green, non-polar aromatic residues are highlighted in green.