Supplementary Information

Characterization of a novel species of adenovirus from Japanese microbat and role of CXADR as its entry factor

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Myotis macrodactylus Vespertilio sinensis

Fig. S1 Bat species from which we isolated BtAdVs. BtAdV-Mm32 and Vs9 were isolated from *Myotis macrodactylus* or *Vespertilio sinensis*, respectively. We took all these photos.
**BtAdV-Mm32 (31,750 bp)**

**BtAdV-Vs9 (31,218 bp)**

**Fig. S2** Genome organization of BtAdV-Mm32 and -Vs9. The total genome length is shown next to the strain name. Each arrow indicates protein-coding ORF and its direction. Black square shows ITR.
Fig. S3. Expression of canine (c) CXADR. cCXADR was not detected in three lines of CXADR-KO cells, unlike wild type (WT) cells, by a western blot analysis using a mouse anti-CXADR monoclonal antibody (CXADR E1). Plasmid-expressed cCXADR was used as a positive control (PC). ACTB was used as a loading control in each lane with the same amount of sample. This gel/blot was shown in Figure 6A (A). Multiple exposure images of the full-size gels/blots are also shown (B, C).
Fig. S4. Expressions of canine (c), human (h), or bat (b) CXADRs. They were rescued in these addback or transduced cells as revealed by western blot analysis using a mouse anti-CXADR monoclonal antibody (CXADR E1). Plasmid-expressed cCXADR was used as a positive control (PC) ACTB was used as a loading control in each lane with the same amount of sample. This gel/blot was shown in Figure 7A (A). Multiple exposure images of the full-size gels/blots are also shown (B, C).
Table S1. Genomic loci of BtAdVs genes and probable functions of their coding proteins.

| Gene   | Genomic position (nt) | Function                          |
|--------|-----------------------|-----------------------------------|
|        | BtAdV-Mm32            |                                   |
| E1A    | 536-1034, 1118-1278   | 462-1014, 1094-1293               | Transcriptional activator         |
| E1B     | 1424-1972             | 1466-1984                         | Small T-antigen                  |
| E1Bl    | 1786-3165             | 1828-3165                         | Large T-antigen                  |
| pIX     | 3231-3551             | 3231-3521                         | Minor capsid protein             |
| IVa2    | 3575-4890, 5190-5202  | 3555-4882, 5161-5173              | DNA packaging                    |
| Pol     | 4675-8094, 12623-12631| 4646-8053, 12581-12589            | DNA polymerase                   |
| pTP     | 7590-9770, 12623-12631| 7912-9732, 12581-12589            | DNA terminal protein             |
| 52K     | 9697-11010            | 9757-10935                        | DNA packaging protein            |
| pHIIa   | 10886-12610           | 10802-12553                       | Minor capsid protein             |
| Penton  | 12657-14087           | 12613-14046                       | Major capsid protein             |
| pVII    | 14119-14523           | 14074-14484                       | Minor core protein               |
| pV      | 14589-15875           | 14551-15780                       | Minor core protein               |
| pX      | 15901-16110           | 15805-16011                       | Minor core protein               |
| pVI     | 16159-16959           | 16060-16806                       | Minor capsid protein             |
| Hexon   | 17029-19755           | 16879-19605                       | Major capsid protein             |
| Protease| 19767-20387           | 19607-20224                       | Endopeptidase                    |
| Protein  | Start-Stop A | Start-Stop B | Function                                      |
|----------|--------------|--------------|-----------------------------------------------|
| DBP      | 20393-21847  | 20264-21622  | DNA binding protein                           |
| 100K     | 21860-23911  | 21634-23718  | Hexon assembly protein                        |
| 33K      | 23793-23937, 24050-24399 | 23588-23744, 23857-24212 | DNA packaging protein                         |
| 22K      | 23793-24260  | 23588-23986  | DNA packaging protein                        |
| pVIII    | 24403-25071  | 24214-24894  | Minor capsid protein                          |
| E3       | 25438-26586  | 25274-26425  | Modulates immune response                     |
| U exon   | 26559-26762  | 26428-26595  | Viral replication                             |
| Fiber    | 26761-28428  | 26594-28144  | Major capsid protein                          |
| E4-ORF6/7| 28442-28684, 29814-29861 | 28166-28387, 29121-29168 | Transcriptional activator                     |
| E4-34K   | 28685-29467  | 28389-29168  | Double strand break repair                    |
| E4-ORF4  | 29469-29861  | 29170-29550  | Induced cell death                            |
| E4-ORF3  | 29840-30214  | 29553-29924  | Shut-down of cellular protein                 |
| E4-ORF2  | 30394-30753  | 29905-30303  | Unknown                                       |
| E4-ORF1  | 30845-31318  | 30391-30783  | Oncogenicity                                  |
Table S2. Amino acid identity of BtAdV-Mm32 compared to other BtAdVs and CAdVs.

| Viruses | Amino acid identity to BtAdV-Mm32 (%) |
|---------|--------------------------------------|
|         | E1A       | E1Bs      | E1Bl      | pIX       | 1Va2      | Pol       | pTP       | 52K       | pIIa      | Penton    | pVII      | pV        | pX        | pVI       | Hexon     | Protease  | DBP       | 100K      | 33K       | 22K       | pVIII     | U exon    | Fiber     | E4-34K    |
| BtAdV-Vs9 | 41  | 42  | 50  | 53  | 72  | 74  | 80  | 74  | 76  | 83  | 77  | 59  | 83  | 72  | 87  | 78  | 70  | 73  | 59  | 53  | 75  | 65  | 42  | 50  |
| BtAdV-A   | 95  | 97  | 98  | 95  | 98  | 99  | 98  | 99  | 99  | 100 | 98  | 96  | 100 | 95  | 100 | 98  | 99  | 98  | 98  | 99  | 99  | 100 | 98  | 98  |
| BtAdV-B   | 46  | 47  | 53  | 48  | 75  | 74  | 84  | 75  | 78  | 82  | 71  | 60  | 84  | 67  | 86  | 80  | 70  | 78  | 63  | 56  | 80  | 74  | 48  | 49  |
| BtAdV-C   | 21  | 25  | 25  | 42  | 64  | 60  | 64  | 59  | 52  | 65  | 34  | 30  | 58  | 50  | 72  | 65  | 43  | 56  | 43  | 43  | 56  | 32  | 25  | 26  |
| BtAdV-D   | 39  | 17  | 25  | 41  | 63  | 57  | 61  | 59  | 50  | 63  | 39  | 22  | 55  | 45  | 70  | 58  | 53  | 54  | 60  | 41  | 54  | 43  | 26  | 27  |
| BtAdV-E   | 35  | 23  | 25  | 31  | 60  | 56  | 58  | 55  | 52  | 65  | 46  | 21  | 56  | 42  | 71  | 59  | 48  | 55  | 52  | 38  | 56  | 32  | 24  | 24  |
| BtAdV-F   | 20  | 23  | 27  | 42  | 58  | 59  | 52  | 55  | 51  | 63  | 46  | 25  | 57  | 44  | 69  | 56  | 45  | 53  | 53  | 34  | 56  | 39  | 23  | 26  |
| BtAdV-G   | 48  | 58  | 63  | 60  | 79  | 78  | 88  | 82  | 85  | 89  | 84  | 72  | 85  | 80  | 90  | 88  | 79  | 81  | 78  | 67  | 86  | 74  | 50  | 63  |
| CAdV1     | 44  | 45  | 50  | 52  | 72  | 74  | 85  | 77  | 78  | 84  | 74  | 60  | 83  | 71  | 86  | 78  | 66  | 74  | 57  | 60  | 76  | 80  | 45  | 49  |
| CAdV2     | 40  | 47  | 51  | 54  | 76  | 75  | 86  | 78  | 80  | 83  | 74  | 62  | 82  | 73  | 86  | 82  | 66  | 75  | 59  | 58  | 80  | 80  | 50  | 50  |
Table S3. Amino acid identity of BtAdV-Vs9 compared to other BtAdVs and CAdVs.

| Viruses     | E1 A | E1Bs | E1Bl | pIX | Iva2 | Pol | pTP | 52K | pIIa | Penton | pVII | pV  | pX  | pV1 | Hexon | Protease | DBP | 100K | 33K | 22K | pVIII | U exon | Fiber | E4-34K |
|-------------|------|------|------|-----|------|-----|-----|-----|-----|--------|------|-----|-----|-----|-------|----------|-----|------|-----|-----|------|-------|-------|-------|
| BtAdV-Mm32  | 41   | 42   | 50   | 53  | 72   | 74  | 80  | 74  | 76  | 83     | 77   | 59  | 83  | 72  | 87    | 78       | 70  | 73   | 59  | 75  | 65   | 42    | 50    |
| BtAdV-A     | 43   | 46   | 50   | 52  | 72   | 74  | 80  | 75  | 76  | 83     | 77   | 58  | 83  | 70  | 87    | 79       | 70  | 72   | 60  | 53  | 76   | 65    | 43    | 49    |
| BtAdV-B     | 49   | 44   | 58   | 54  | 75   | 76  | 84  | 73  | 79  | 86     | 85   | 61  | 85  | 64  | 85    | 82       | 69  | 75   | 81  | 63  | 79   | 72    | 40    | 45    |
| BtAdV-C     | 22   | 24   | 28   | 50  | 61   | 60  | 62  | 56  | 50  | 67     | 35   | 27  | 61  | 44  | 72    | 61       | 53  | 53   | 36  | 50  | 53   | 33    | 25    | 26    |
| BtAdV-D     | 33   | 21   | 26   | 31  | 64   | 57  | 58  | 62  | 50  | 65     | 37   | 24  | 57  | 45  | 70    | 60       | 53  | 55   | 62  | 37  | 49   | 51    | 25    | 27    |
| BtAdV-E     | 26   | 21   | 24   | 30  | 60   | 56  | 58  | 59  | 52  | 64     | 35   | 23  | 57  | 47  | 71    | 59       | 44  | 54   | 58  | 36  | 51   | 47    | 19    | 26    |
| BtAdV-F     | 27   | 18   | 26   | 35  | 58   | 59  | 54  | 61  | 50  | 62     | 44   | 23  | 55  | 50  | 69    | 58       | 48  | 53   | 58  | 36  | 53   | 42    | 24    | 25    |
| BtAdV-G     | 43   | 38   | 54   | 62  | 74   | 75  | 83  | 77  | 78  | 84     | 81   | 66  | 81  | 72  | 85    | 81       | 73  | 75   | 64  | 62  | 77   | 63    | 44    | 49    |
| CAdV1       | 43   | 45   | 55   | 56  | 75   | 76  | 82  | 75  | 77  | 86     | 76   | 65  | 80  | 69  | 85    | 82       | 64  | 73   | 71  | 67  | 69   | 42    | 48    |       |
| CAdV2       | 44   | 50   | 58   | 55  | 75   | 77  | 83  | 77  | 78  | 86     | 80   | 66  | 82  | 71  | 85    | 84       | 64  | 74   | 56  | 63  | 75   | 70    | 43    | 46    |
Table S4. Primer sets used for quantification of viral attachment by qPCR.

| Primer name | 5'-3'            |
|-------------|------------------|
| rt Mm32 pol F | TCTCATTGGAAGGGGCAAC |
| rt Mm32 pol R | TGGTCAGCGGCTACCATT |
| rt Vs9 pol F   | CCTCCCCAAAGTGCCTAC TTC |
| rt Vs9 pol R   | GTTGACCTACACGTGGCA |
| rt CAV1 pol F  | GCATAAAAAAGGGCTTCAGCG |
| rt CAV1 pol R  | TTTGCGTAAGCGTACCCACCC |
| rt CAV2 pol F  | TGGCGGTAGGTCATAGAGGT |
| rt CAV2 pol R  | CACTAAGCATTTCCGCCCGTG |
| rt ACTB F      | GGACCTGACCGACTACCTCA |
| rt ACTB R      | GTCCAGGGCCACATAACACA |